OUTBREEDING DEPRESSION AND INHERITANCE IN THREE GENERATIONS OF GEOGRAPHICALLY DISTINCT SOUTHEAST ALASKA COHO SALMON (*ONCORHYNCHUS KISUTCH*) POPULATIONS

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A

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Abstract

I observed no fitness losses among F_2 hybrids of three Southeast Alaska coho salmon (*Oncorhynchus kisutch*) populations relative to parental controls. Marine survival did not differ among groups in one generation, but was greater for hybrids than controls in another, although the power of these tests was low. Increases in fluctuating asymmetry, which can signal losses in fitness, were not observed. Line cross analyses of length suggested additive and additive plus dominance gene action, and two of three analyses suggested epistasis. In contrast, meristic characters exhibited little variability; and in most cases tests failed to reject a simple additive model. Half- and full-sib analyses provided no evidence of quantitative genetic variation for any trait although the power to detect these effects was low. Comparisons of population divergence measured by quantitative traits (Q_{ST}) and molecular markers (F_{ST}) that length is an adaptive trait and that bilateral meristics are highly conserved. Although we did not observe losses in fitness, the power of our tests was low, the among-population differences were unique to our experiment and so results of this study should be interpreted with caution.

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Acknowledgments

I am a hybrid of two distinct human populations, and I thank my wonderful parents Bill Dann and Jenny Alowa for giving heterosis a shot and for all they have done for others in their lives. This thesis would not have been completed without the support of Valli Peterson, the love of my life, and I thank her for all of her support. Our dogs Dolly and Cisco sacrificed many a walk so that this thesis could be completed and I appreciate their patience and foot warming on late nights. I acknowledge my adviser Dr. Tony Gharrett for being very generous with his time and knowledge and for working with me at a distance while this was completed in Anchorage. Similarly, my other committee members Drs. Jeff Hard and Bill Smoker were very generous with their quantitative genetics and technical writing expertise. This research was funded by the National Marine Fisheries Service, Northwest Fisheries Science Center, Seattle, Washington. During the course of my graduate studies I received financial support from the Sitnasauk Foundation, the Bering Straits Foundation, the Alaska Fish and Wildlife Safeguard/Mil Zahn Memorial Scholarship, the Glenn Carrington Memorial Scholarship Fund, and the Austin Cooley Talent Grant. The St. Lawrence Island community and the Savoonga Native Corporation generously offered their support and blessings for an earlier study that was ultimately not funded. Detlef Buettner of the Alaska Department of Fish and Game (ADF&G) Mark, Tag and Age Laboratory provided valuable assistance in the retrieval of coded-wire tag data. Stephanie Walden initiated me to genetic laboratory techniques and helped me start putting fish to parent. Members of the Gharrett Laboratory Katie Palof, Michael Garvin, Lisa Kamin, Jesse Echave, Sharon Hall and Rachel Riley all provided academic, technical and moral support at times.

General Introduction

This thesis presents work that investigates the effects of hybridization of coho salmon (*Oncorhynchus kisutch*) populations on fitness and the quantitative genetics of traits over three generations. Pacific salmon (*Oncorhynchus* spp.) are keystone species in their natural ecosystems and for human societies. A component of the success of Pacific salmon is the impressive variety and degree of adaptations to the differing environments encountered across their range and life history stages. The distinct island nature of salmonid freshwater spawning systems, combined with the propensity of Pacific salmon to home to natal streams, serves to isolate populations from one another and limit the exchange of genetic information. Because the range of salmon life histories and environmental conditions varies across the Pacific Rim, different selection regimes encountered by these populations generate local adaptation. Local adaptation can be explained as genetic alteration of a population that produces phenotypes that succeed in the local environment (Orr 2005). Sustaining this genetic diversity is important to the long term viability of these species and the fisheries that depend upon them because the genetic diversity and associated phenotypic variation may serve as a buffer against interannual or global environmental changes or anthropogenic disturbances (Wang et al. 2002; Utter 2004; Carlson and Seamons 2008). These differences are important at geographic scales of large stock complexes (Hilborn et al. 2003) and small populations (Geiger et al. 1997). Because neither Pacific salmon population nor evolutionary dynamics are static, it is important to maintain a diverse array of populations.

Outbreeding depression (OBD) is the loss in fitness that can occur to the hybrids of distantly related populations (Lynch 1991) and is a conservation concern for Pacific salmon. Effects of OBD may not be manifested until the second or later generations. Due to local adaptation and population divergence, and the potential for non-native gene introgression consequent to common salmonid resource management practice, understanding the process that results in fitness losses due to the erosion of this divergence is important. Relatively few studies have examined the effects of hybridization through multiple generations, which may be necessary to observe OBD.

Other studies designed to examine OBD have had mixed results (McClelland and Naish 2007).

Many of the kinds of traits that are important in local adaptation result from the actions of multiple loci. Such traits are referred to as polygenic or quantitative traits. The focus of quantitative genetic analysis involves partitioning the phenotypic variation observed for a quantitative trait into its genetic and non-genetic sources (Lynch and Walsh 1998). This type of analysis may be useful for the selection of traits desirable in artificial culture, in addition to improving our understanding of the evolutionary processes that shape populations facing ecological or anthropogenic change. Estimating heritabilities is traditionally accomplished with a pedigree-based approach that evaluates the covariance of trait values of genetically related individuals. In practice, the approach is often used to evaluate the significance of different sources of genetic variance associated with the sire and dam components in an analysis of variance of their full- or half-sib progeny. At a population scale, the composite effects of different types of gene action on quantitative traits can be evaluated using the known interaction of genes that exist when members of different populations are crossed. This approach is known as line cross analysis.

This thesis presents an uncommon combination of population and quantitative genetics. Population genetic analyses examined the molecular genetic divergence among these populations, thereby providing a context for tests of OBD and evaluating the importance of natural selection that underlies the quantitative traits we measured. Both traditional quantitative genetics approaches and line cross analysis were used to evaluate the inheritance of quantitative traits and the type of genetic action that best explains these traits. This combination provides for an interesting and detailed examination of salmonid local adaptation, the effects of local adaptation on fitness, and the evolutionary processes that contribute to population divergence.

I organized this work into two chapters. Chapter 1 investigates the fitness effects of hybridizing distinct populations. This study was designed to test for the fitness loss that resulted from OBD over multiple generations of hybridization. Fitness was measured

in two ways: marine survival and developmental stability as measured by the fluctuating asymmetry of bilateral traits. Because bilateral meristic traits were used in tests of fitness, the variance observed for these traits and for length was also partitioned to estimate the influence of sex, year, population, and type of cross (control or hybrid) on trait distributions. In Chapter 2, I investigated quantitative genetic variation in length and the bilateral meristics measured in this study. I used two complementary approaches: traditional heritability estimation, which partitions observed phenotypic variance into different genetic and environmental sources, and line cross analysis, which assesses the composite effects of different modes of gene action on trait distributions and population divergence. Measures of population divergence based upon quantitative traits (Q_{ST}) and neutral molecular markers (F_{ST}) were also compared to evaluate the degree and direction of selection. Both chapters are formatted for submission to the Transactions of the American Fisheries Society.

Many individuals contributed to the work presented in the following pages. Drs. Robin Waples, Jeff Hard, Bill Smoker and A.J. Gharrett hatched the idea for this study and initiated the process of experimental design and implementation. Drs. Hard, Smoker and Gharrett oversaw the study through multiple generations of coho salmon and cohorts of graduate students. Karla Bush (formerly Karla Granath) was the first graduate research assistant associated with this study and oversaw the initial crossing of broodstock, rearing of juveniles and the analyses of embryo development and survival for the first generation of crosses. Stephanie Walden was the second graduate research assistant associated with this study and oversaw the initial genotyping of the original broodstock (BY94) and their offspring (BY97) and the pedigree construction of these two generations. Sharon Hall contributed to much of the field sampling and collection of morphological data as a research staff member of the Gharrett Laboratory. Ivan Wang oversaw the sampling and crossing of BY97 returns, the rearing and release of BY00 juveniles and the logistics of maintaining the University's Macaulay Salmon Broodstock Laboratory at the Douglas Island Pink and Chum, Inc. (DIPAC) Hatchery. Many other graduate assistants from the University of Alaska Fairbanks' School of Fisheries and Ocean Sciences contributed to

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Chapter 1:

Outbreeding depression after two generations of hybridizing Southeast Alaska coho salmon populations?[1]

Abstract

We observed no losses in fitness among second-generation hybrids of three Southeast Alaska coho salmon (*Oncorhynchus kisutch*) populations as compared to parental controls. Divergence among these populations measured from neutral molecular markers was highly significant (F_{ST} = 0.028; $P < 0.0001$). Marine survival did not differ among parental groups (P = 0.60), among F_1 groups (P = 0.75), among F_2 groups (P = 0.31), between parental and hybrid groups (P = 0.88), among the hybrid groups (P = 0.07), or between parental and F_2 groups (P = 0.59) in analysis of the brood year 2000 generation. Marine survival of F_1 hybrids in brood year 1997 exceeded that of parental controls (P = 0.004), but did not differ among parental groups or among F_1 groups, although the power of the latter tests was low. Length differed among years, populations, sexes, and cross types (parental, F_1 hybrids, F_2 hybrids), but differences among cross types reflected among-population differences. In contrast, bilateral meristics exhibited little variability (CVs = 0.02 – 0.09). The differences observed among years for meristics likely reflect effects of the different environments experienced. Measures of fitness losses of hybrids relative to parental controls that were based upon the increased fluctuating asymmetry (FA) of bilateral meristics yielded only one significant result, and in many cases hybrids exhibited less FA than parental controls. The little variability we observed for meristic characters suggests strong genetic canalization for these traits. Although we did not observe losses in fitness as measured by marine survival and FA, the power of

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[1] Dann, T.H., J.J. Hard, A.J. Gharrett, and W.W. Smoker. 2009. Outbreeding depression after two generations of hybridizing Southeast Alaska coho salmon populations? Prepared for submission to the Transactions of the American Fisheries Society.

each of our tests was low, the among-population differences were unique to this experiment and so results of this study should be interpreted with caution.

Introduction

Pacific salmon are keystone species to the ecology of their environment and society. The propensity of salmon to home to their natal stream, in concert with local adaptation to different selective forces across their range coupled with genetic drift, results in genetically divergent populations across their range. The value of this diversity has been acknowledged (Wang et al. 2002, Hilborn et al. 2003), especially in the southern portion of Pacific salmon range where greater threats to their viability exist (Gustafson et al. 2007). Hatcheries have been widely used to create recreational sport fisheries, supplement depressed populations, and create common property harvest opportunity. Through stock translocation, introduction of non-local broodstock, and straying, hatchery-produced fish have the potential to remove barriers to reproduction that can degrade the genetic health of wild populations if non-native genes introgress into wild populations (reviewed in Waples 1991). Outbreeding depression is the loss in fitness caused by the hybridization of distantly related populations (Lynch 1991) and is a conservation concern for salmon (Gharrett et al. 1999, McClelland and Naish 2007).

Outbreeding depression can occur through two mechanisms, which can co-occur. The first mechanism is known as extrinsic outbreeding depression and involves the interaction of alleles at a locus and the environment. It reflects the loss of local adaptation and is a mismatch between average gene effects and the local environment. This mechanism is possible in the F_1 generation of hybrids and beyond. The second mechanism is known as intrinsic outbreeding depression and involves the interaction of alleles at different loci. It results from the disruption of coadapted gene complexes (i.e., positive epistatic gene interactions with respect to fitness) and is generally not possible until the F_2 generation of hybrids (i.e., after independent assortment and sometimes recombination). Because these mechanisms differ, results of hybridization observed in the F_1 generation can not predict results of hybridization in later generations. Relatively

few studies have investigated outbreeding depression through two generations of hybridization, and results from those that have are mixed (Edmands 2007; McClelland and Naish 2007).

Defining direct measures of fitness is a problem when conducting hybridization studies. Fitness is the ability of an organism to pass its genetic information on to future generations (Hedrick 2005). Marine survival of marked or tagged fry is a meaningful and direct measure of fitness for Pacific salmon and has a long history of use (e.g., Bams 1976; Smoker et al. 2004). Other indices of fitness have been suggested that would document fitness changes as the populations change rather than measuring fitness retrospectively. The fluctuating asymmetry (FA) of bilateral traits has been proposed as such an index of fitness. Fluctuating asymmetries are small, random departures from symmetry in bilaterally symmetrical traits (Palmer and Strobeck 2003). These departures from symmetry are deviations from the development of an 'ideal' form of a trait as would be expressed when development is stable. Developmental stability is the outcome of canalization, the term applied to the buffering of phenotypic variation influencing trait development (Waddington 1942). Canalization can be categorized as the buffering of either genetic or environmental perturbations. A deterioration of the canalization process may lead to losses in fitness in the presence of these perturbations. Studies have assessed and analyzed different indices of FA (Palmer and Strobeck 1986) and their utility as fitness measures (Moller 1997). The numerous measures of FA are all based upon differences in count or size of bilateral meristic characters and have been used as indices of fitness in a number of salmonid populations (Leary et al. 1985; Campbell and Emlen 1996; Moran et al. 1997; Johnson et al. 2004). Application of analyses to variation in FA has had mixed success. Criticisms of such applications are the failure of studies to adopt a uniform methodology of estimating developmental stability through FA, the relatively unknown effect of genotype and environment on developmental stability as measured by FA, and the often small signal that suggests a weak relationship between organismal stress and FA (Lens et al. 2002; Van Dongen 2006).

Our objective was to study the effects of hybridizing three Southeast Alaska coho salmon populations originating across a geographic distance meaningful to resource managers. The primary questions were: (1) Does outbreeding result in a loss of fitness in F_1 or F_2 hybrids? (2) Are there differences in the mean or variance of the FA of bilateral meristics between controls and hybrids? and (3) What factors explain the variations of length and bilateral meristics of these coho salmon?

Methods

Field Methods

We crossed coho salmon from Neets Bay, Hidden Falls, and Gastineau (also known as Macaulay or DIPAC) hatchery stocks, which had been separately derived from three geographically different Southeast Alaskan drainages. The Neets Bay lineage was established in 1982 and originated from Indian Creek, a tributary of the Chickamin River, which is near Ketchikan, Alaska. The Indian Creek stock is the southernmost population in the study and is fed by high mountain streams. The Hidden Falls population was established in 1985 and originated from an unnamed, lake-fed stream that enters Deep Cove on southern Baranof Island. The Gastineau population was also established in 1985; it originated from Montana Creek, a tributary of the Mendenhall River, which is near Juneau, Alaska and is the northernmost population in the study. These populations had been cultured for only three to four generations at the onset of our study, and serve as proxies for the corresponding wild source populations. Similarly, although it was not possible to compare hybrids in each parental environment, all experimental crosses experienced the same rearing conditions. The standardized rearing conditions employed technology similar to that used at each of the donating hatcheries. This included Heath incubators, manufactured semi-moist diets at recommended rations, and the release of one-check smolts to the estuary in the spring.

We flew gametes from Hidden Falls and Neets Bay hatcheries to Gastineau Hatchery on 6 November 1997 and spawned them on the same date with a full-sibling design to create brood year 1997 (BY97). We used gametes from 50 males and 50

females from each hatchery as the initial broodstock, but after removing individuals that tested positive for bacterial kidney disease (*Renibacterium salmoninarum*), we included between 32 and 45 males and 32 and 45 females from each hatchery as broodstock. We created nine different crosses in the F_1 generation: three replicate parental controls and six reciprocal F_1 hybrid crosses between the parental sources (Table 1.1 and Appendix Table A1). We reared these crosses in similar incubation and raceway environments (described in Granath et al. 2004), released them to sea (N = 54,251), and captured them as returning adults (N = 156). On 16 November 2000, we crossed the mature BY97 adults in a half-sibling design to produce the 15 F_2 experimental groups of brood year 2000 (BY00): three replicate controls (parentals), six replicate reciprocal F_1 crosses, and six reciprocal F_2 hybrid crosses (Table 1.2 and Appendix Table A2). We reared these crosses in incubation and raceway environments that were similar to those of the first generation, released them to sea (N = 96,260), and captured them as returning adults (N = 1,026).

We tagged returning BY00 fish with Alaska Department of Fish and Game (ADF&G) numbered jaw tags, collected heart tissue for DNA analysis, and froze fish for future morphological analysis. We preserved the heart tissue (Seutin et al. 1991) and stored it at -20°C until DNA isolation. We obtained mid-eye fork length (MEFL), and meristic counts from thawed fish: counts of pectoral (P) and pelvic (V) fin rays, branchiostegals (B), and both upper and lower (U and L) gill rakers on the first and second (1 and 2) gill arches. We separated fin rays with scalpels for counting and removed entire gill arches from fish for gill raker counts. We included the middle gill raker on each arch with the count of the lower gill rakers. We took two independent counts on each trait for quality control, and resolved the few discrepancies by discussion between observers and additional counting until a consensus was reached. Although this approach precluded estimates of measurement error, it produced very accurate data. We removed the snouts of tagged fish to isolate coded-wire tags (CWT), which were decoded by the ADF&G Mark, Tag and Age Laboratory (Juneau, AK) under their standard quality-controlled procedures.

Laboratory Methods

We isolated total genomic DNA with the Puregene® DNA purification protocol for fish tissue (Gentra Systems, Valencia, CA). We PCR-amplified microsatellite loci in a Stratagene (La Jolla, CA) 96 Robocycler™. The reaction mixtures were 10 μL volumes that included approximately 1 unit of *Taq* polymerase and final concentrations of: 1X PCR buffer (50 mM KCl, 10 mM Tris HCl pH 8.3), 0.25 mM $MgCl_2$, 0.125 mM of each deoxynucleotide triphosphate (dNTP), approximately 0.05 to 0.10 μg DNA template, and 0.35, 0.4, and 0.04 μM of forward, reverse, and labeled primer, respectively, overlaid with mineral oil. We used six loci in this study: Oki1, Oki10, Oki16, and Oki20 (Smith et al. 1998); Ots101 (Small et al. 1998); and Ots208 (also known as OtsG68; Greig et al. 2003; Williamson et al. 2002, respectively). The PCR conditions and locus information are listed in Table 1.3. We denatured amplified DNA product by adding an equal volume of stop buffer (95% formamide, 0.1% Bromophenol Blue), heating it for 3 minutes at 95°C, and cooling it rapidly on ice.

We loaded 1 μL of PCR product from each individual onto 0.25 mm polyacrylamide denaturing gels composed of 6% polyacrylamide gel made from 40% 19:1 acrylamide/bisacrylamide solution, 7 M urea, and 5X TBE (TBE is 90 mM tris-boric acid and 2mM EDTA, pH 7.5) in a reaction catalyzed by ammonium persulfate and TEMED (N,N,N',N'-tetramethylethylenediamine). We separated alleles by size by gel electrophoresis that was performed on LI-COR (Lincoln, Nebraska) automated sequencers (LongReadR 4200™ and 4300 System ™) in 1X TBE buffer (0.09M Tris-Borate, 2mM EDTA, pH 8.3) with running conditions 1500 V (approximately 40 W and 40 mA) and 45°C plate temperature. We scored allele sizes with Saga^{GT} (Ver. 3.2.1, LI-COR) analysis software by comparing bands with IRD700 or IRD800 standard ladders (LI-COR, Biotechnology Division).

Statistical Methods

Allele frequency analysis.—We examined microsatellite allele frequencies of BY94 fish to assess how genetic variation was partitioned among the three source populations. Four loci were used in this analysis: Ots101, Oki1, Oki10, and Oki16.

Although these loci were chosen to assign parental pairs and not describe population structure, they provide insight into how genetic variation is partitioned in these populations. We tested for Hardy-Weinberg equilibrium by locus and population using the program GENEPOP version 3.4 (Raymond and Rousset 1995). We calculated fixation indices (*F*-statistics) by the Weir and Cockerham method in the program Arlequin version 3.1 (Excoffier et al. 2005).

Survival.—We defined survival as the proportion of released smolt that were identified from coded-wire tags in the fishery or recovered as maturing adults at the Sheep Creek hatchery facility. We used microsatellite genotypes to assign maturing offspring to parental pairs by exclusion analysis based on known parent matings (PROBMAX Version 1.3; Danzmann 1997). We determined types of crosses from assigned parent pairs and verified them by CWT (present in 96% of the fish). We identified adults reported from sport and commercial harvests from the ADF&G's CWT database (http://tagotoweb.adfg.state.ak.us/CWT/reports/user_login.asp) and included them with fish recovered at Sheep Creek for analysis. After the returning fish had been assigned to families, we used log-likelihood ratio tests (*G*-test; Sokal and Rohlf 1995) to test for homogeneity of survival once the types of cross that produced each fish were determined. We used a hierarchical approach to test the homogeneity of survival: (1) among the parental groups ($P_i = P_j = P_k$); (2) among the F_1 groups ($F_{1ij} = F_{1ik} = F_{1jk}$); (3) among the F_2 groups ($F_{2ij} = F_{2ik} = F_{2jk}$); (4) between parental control and hybrid groups ($P = F_1 + F_2$), and (5) between the hybrid groups ($F_1 = F_2$), where i, j, and k denote the sources of lineages contributing to the crosses.

We also used log-likelihood ratios to test for homogeneity of survival among BY97 return data queried from the ADF&G's CWT database. We used a similar hierarchical approach to test the homogeneity of survival: (1) among the parental groups ($P_i = P_j = P_k$); (2) among the F_1 groups ($F_{1ij} = F_{1ik} = F_{1jk}$), and (3) between parental control and F_1 hybrid groups ($P = F_1$), where i, j, and k denote the sources of lineages contributing to the crosses. We used the PROC POWER procedure in SAS to examine power curves for tests of homogeneity of survival (SAS Institute Inc. 2004).

Trait analysis.—We examined Pearson correlation coefficients among the BY00 character data, and also among character data from parental controls from all brood years. We corrected tests of significance of correlations for multiple testing with a sequential Dunn-Sidak procedure. We regressed length on meristic data to test whether or not length should be included as a covariate in models that partitioned meristic variance; these regressions were performed on BY00 and all brood year data separately. We corrected length regression results for multiple testing with a sequential Dunn-Sidak correction for 14 tests (14 bilateral traits). None of the regressions was significant in the BY00 data, and only one meristic character had a small negative relationship with length in the data from parental controls from all brood years (UR2, $P < 0.001$; Appendix Table A3), so we did not incorporate length as a covariate into variance partitioning models. We tested the equality of means and variances of BY00 reciprocal crosses, and corrected these tests for multiple testing with the sequential Dunn-Sidak method for 15 tests (14 bilateral traits and length). We tested normality and homogeneity of variances of character data grouped according to models specified below to verify model assumptions.

Analysis of variance.—We used two approaches to analyze the variation of meristic, length, and FA data: analysis of variance (ANOVA) and the nonparametric Kruskal-Wallis test. Since the effects of generation of parental and hybrid cross and population source overlap among the three populations and are confounded, we conducted two analyses, which independently tested the effect of parental origin (hereafter referred to as the stock-specific model) and the generation of hybrid cross (hereafter referred to as the hybrid-cross models). These models incorporated character data from multiple (BY94, BY97, and BY00) and single (BY00) years, respectively.

Analysis of variance ordinarily assumes that the data are normally distributed and that the variances of groups are homogeneous (homoscedasticity). However, few of our traits were normally distributed and could not be normalized by transformations. Similarly, we observed heteroscedasticity in the stock-specific model data set (i.e., two characters when grouped by population, three when grouped by brood year, and three

when grouped by sex) and the hybrid-cross model data set (i.e., four characters when grouped by sex and one when grouped by cross) after correcting for multiple testing.

We report primarily the results of the Kruskal-Wallis tests because of the distribution of our data. However, the ANOVA and Kruskal-Wallis test produced similar results, and few of the possible ANOVA interaction terms were significant. Because the Kruskal-Wallis test measures differences of a single variable across two or more independent groups, it is analogous to a one-way ANOVA, but does not test the interactions of effects. For effects tests that included just two groups (i.e., sex), the Kruskal-Wallis test reduces to the Mann-Whitney U test, the nonparametric analog of the two-sample *t*-test (Sokal and Rohlf 1995). We conducted all tests with SYSTAT 11 (SPSS 2004).

The stock-specific model tested the effects of sex, year, and parental source. Because we were interested in the effect of source population, we did not include character data from hybrid crosses in this model. We used only the character data from the three parental control crosses, but included data from all three generations. This model was:

$Y_{ijkl} = \mu + Sex_i + Year_j + Pop_k + e_{ijkl}$

where Y_{ijk} is the observed character, Sex_i is the effect of the i^{th} sex, $Year_j$ is the effect of the j^{th} year, Pop_k is the effect of the k^{th} population, and e_{ijkl} is random error. The ANOVA model that we tested also included interaction terms (Sex*Year, Sex*Pop, Year*Pop, and Sex*Year*Pop).

The purpose of the hybrid-cross model was to examine the influence of the control and hybrid crosses (parental control, F_1 hybrid, F_2 hybrid) on morphological characters. The hybrid-cross model tested the effects of sex and cross on these characters. We included character data from only the BY00 generation, because it was the single brood year for which we had data from all three types of cross and which shared similar rearing environments. These models included the source of parental cross as an effect rather than experimental group (e.g., GGGG and NNNN correspond to offspring from control (parental) crosses between F_1 returns of control Gastineau and Neets Bay crosses,

respectively; GGNN are the offspring from the F_1 hybrid cross between Gastineau female and Neets Bay male sources; and GNNG are the offspring of the F_2 cross between returns of F_1 crosses between Gastineau × Neets Bay females and Neets Bay × Gastineau males [see Tables 1.1 and 1.2]). We did this to account for differences in parental controls that are incorporated into the model. A single model that incorporates all three parental crosses and their F_1 and F_2 hybrids that is based on population source (G, H, or N) would confound parental source and specific hybrid crosses. Consequently, we evaluated six separate models, each of which included the two parentals and one of the types of hybrid (F_1 or F_2, but not both), and compared both hybrids to their parental crosses (e.g., as described above: the two parentals GGGG and NNNN and their F_1 hybrid GGNN or their F_2 hybrid GNNG). This model was:

$$Y_{ijk} = \mu + Sex_i + Cross_j + e_{ijk}$$

where Y_{ijk} is the observed character, Sex_i is the effect of the i^{th} sex, $Cross_j$ is the effect of the j^{th} cross-type, and e_{ijk} is random error. When the cross term was significant, we used a post-hoc test to evaluate the null hypothesis that the hybrid mean was intermediate to its parental means. The ANOVA model also included the Sex*Cross interaction term.

Fluctuating asymmetry.—We examined all bilateral traits for directional asymmetry (DA) to ensure that FA indices were not artificially inflated (Palmer 1994) and that composite FA indices (CFAs) included only traits that exhibited directionally random asymmetry (Leung et al. 2000). We quantified directional asymmetry as the mean difference between right and left side traits and tested with a two-tailed *t*-test (null hypothesis: mean (R-L) = 0). Rejection of the null hypothesis implies significant DA. We corrected tests for DA for multiple tests with a sequential Dunn-Sidak correction for 7 tests (7 pairs of bilateral traits).

We measured fluctuating asymmetry as $|R - L|$. We performed regressions of the unsigned difference between counts of both sides ($|R-L|$) for each trait against an independent index of body size (length) to test the assumption that observed FA among groups is independent of group body size mean and variance (Palmer 1994). The results of these regressions, which were performed on both BY97 and BY00 data, were not

significant after correcting for multiple tests with a sequential Dunn-Sidak correction for 7 tests (7 pairs of bilateral traits).

Fluctuating asymmetry can be measured in several ways; we evaluated several indices of FA and chose two that were appropriate for our data: FA1 and FA5 (Palmer 1994; Palmer and Strobeck 1986). We also evaluated two indices of FA that correct for size-dependence by individual (FA2) and sample (FA3); however, we did not observe size-dependence and so these indices yielded very similar results to FA1. The index FA1 is a commonly used index of FA because it is the absolute value of asymmetry and is calculated as:

$$FA1_i = \frac{\sum (A_{ijk})}{N}$$

where A_{ijk} is |R-L| for the k^{th} character of the j^{th} individual of the i^{th} population. It is relatively insensitive to outliers but does not correct for differences in character size or number and will be biased if there is strong trait DA or antisymmetry, which describes the case where many individuals in a sample will have a larger trait value on one side of the body while the other members have a larger trait value on the other side (i.e., bimodal trait distribution). The index FA5 estimates the variance between sides and is calculated as:

$$FA5_i = \frac{\sum (A_{ijk})^2}{N}$$

where A_{ijk} is defined as above and is the variance of the difference between R and L when the average difference is zero. This index is powerful at detecting true differences in FA among samples (Palmer and Strobeck 1986). However, it is sensitive to DA, outliers, and character size. The assumption that the mean is zero is appropriate for our analysis because only characters that did not exhibit DA were included in CFAs for further analysis.

Because the observed differences in counts of bilateral meristics are often small, and measures of FA for individual characters only weakly indicate developmental instability (Van Dongen 2006), we adopted the CFA1 index (Leung et al. 2000) to

combine information from all characters that did not exhibit DA into a composite measure of each FA index described above. The index CFA1 is the sum of absolute FA values for all characters for each individual. It is calculated as:

$$CFA_i = \sum_{j=1}^{k} \left| FA_{ij} \right|$$

where *i* is individual, *j* is trait and *k* is number of traits per individual for each index.

We compared means and variances of CFA values of the outbred groups (F_1 in BY97, F_1 and F_2 in BY00) to their two parental sources to test for differences that may have arisen from outbreeding. We tested the equality of CFA variances with Levene's test for homogeneity of variances prior to testing means. We tested the equality of CFA means and variances between each outbred group and each of its two parental sources with a one-tailed *t*-test. We used standard *t*-test formulae for homoscedastic and heteroscedastic conditions (Expression 9.2 and Welch's approximate *t*-test, respectively; Sokal and Rohlf 1995).

Results

Allele frequencies

Oki16 did not conform to Hardy-Weinberg equilibrium expectations ($P = 0.005$) and had an excess of heterozygotes in the Gastineau population (F_{is} = -0.127; Table 1.4). The F_{ST} among the three populations was 0.028 ($P < 0.0001$) and the overall F_{IS} was -0.013 ($P = 0.91$). Pairwise comparisons of F_{ST}s between population pairs suggested that all three stocks differed to a similar extent (Table 1.5).

Survival

We captured and genotyped 943 BY00 fish that we were able to assign to BY97 parents. These fish added 40 adults that did not have CWTs to the experimental fish identified in the ADF&G's CWT database that was previously reported (Smoker et al. 2004). The total recovery of BY00 fish at Sheep Creek Hatchery and in fisheries was 1,778 returning adults from 96,260 smolt that were released (Table 1.6). This total did not include an expansion of the tags that were recovered from random samples of fishery

harvests. We observed no significant differences in survival among parental groups, among F_1 groups, among F_2 groups, between parental and hybrid groups, among the hybrid groups (Table 1.7), or in a separate test, between parental and F_2 generations (P = 0.59). The query of BY97 fish from the ADF&G CWT database resulted in a total recovery of 847 returning adults from 54,251 smolt that were released. We observed a significant difference between parental and F_1 hybrid groups ($F_1 > P$; $P = 0.004$), but not among parental groups or among F_1 groups.

Trait analysis

Six hundred and forty-four BY00, 281 BY97, and 268 BY94 fish of the adults that returned to Sheep Creek were measured for bilateral trait data. Length data were taken from 698 BY00, 281 BY97, and 268 BY94 fish of the total Sheep Creek returns. Most of the models we analyzed included subsets of these data. The stock-specific model included only parental controls: 56 fish from BY97 were measured for length and bilateral data; from BY00 127 fish were measured for length, and 117 for bilateral traits.

Character correlations.—Length did not correlate significantly with any of the bilateral meristic counts in either the hybrid-cross or stock-specific data sets ($r < |0.14|$). In both data groupings each bilateral count was most closely correlated with the count from its complementary structure ($0.35 < r < 0.67$; $P < 0.001$). The gill raker counts on gill arch segments were more closely correlated with other gill raker counts than counts of fin rays on one fin were correlated with counts on other fins (Appendix Tables A4 and A5).

Homogeneity of BY00 reciprocal crosses.—Means and variances of character data for reciprocal crosses in BY00 exhibited some differences. The variances of left pelvic fin rays differed between GHHG and HGGH ($P < 0.001$), and between GNNG and NGGN ($P = 0.002$). In both tests the significant results were due to the low variation in pelvic fin rays; in the case of GHHG/HGGH a total of four non-modal observations (of 91 total) were responsible for the significant results. Furthermore, three of the six reciprocal cross tests of left pelvic fin ray equality exhibited no variation. The variances of right gill rakers of the second lower arch differed between HNNH and NHHN, but this

test was also subject to the high leverage of non-modal observations because of low overall trait variation.

The means of left pectoral fin rays differed between GGNN and NNGG crosses after correcting for multiple tests ($P < 0.001$). Although this result is significant, it represents a mean trait difference of 0.41 fin rays averaged from two observed variates in both crosses. As a result, we pooled reciprocal crosses in further analyses of the hybrid-cross data set.

Analysis of variance

Variation in the stock-specific model.—Mean lengths differed across generations, primarily because the fish used as parents in BY94 crosses were longer (603 mm) than either the BY97 or BY00 fish across all three stocks (556 mm and 561 mm, respectively; $P < 0.001$). Combined across the broods, Gastineau controls were the shortest (540 mm), Neets Bay fish were the longest (622 mm), and Hidden Falls fish had an intermediate length (577 mm) ($P < 0.001$; Figure 1.1). The differences in the sizes of fish derived exclusively from single parental sources persisted over generations. In addition, the average lengths of females exceeded those of males, which do not include precocious males because they were not present; but lengths of males had higher variances (means of 598 mm and 574 mm, $P < 0.01$; variances of 2365 and 4281, $P < 0.001$, respectively for females and males; Table 1.8).

Meristic counts exhibited little variation in the stock-specific model data set (CVs = 0.03-0.06; n = 427-440). Brood year significantly influenced the variation in all but the most conservative traits (i.e., pelvic and pectoral fin rays). Left and right pectoral fin ray counts and some gill raker counts (i.e., LL1, LR1, and UR2) differed among populations, although the magnitude of these differences was small (0.2 and 0.1 fin rays, and 0.2, 0.3, and 0.2 gill rakers, respectively) and had weak significance. Similarly, right pectoral fin rays differed between males and females, although this difference was 0.1 fin rays and was only weakly significant (Table 1.8).

In both the stock-specific and hybrid-cross analyses, standard ANOVA results were very similar to results of the non-parametric tests. Results of interaction terms that

were notable in the stock-specific ANOVA were: a strong population*year influence on length, pectoral fin ray, and left pelvic fin ray counts ($P < 0.001$); a year*sex influence on length ($P < 0.01$); and a population*sex influence on length and left pelvic fin ray counts ($P < 0.01$; Appendix Table A6).

Variation in the hybrid-cross model.—The results of comparisons of the offspring from BY00 parental control and F_1 hybrid crosses were similar to the comparisons between parental control and F_2 hybrid crosses in the hybrid-cross model, except for a few that had only weak significance (Table 1.9).

Lengths differed between sexes and among cross comparisons in all but the Gastineau – Hidden Falls F_1 comparison. Differences among crosses were mostly attributable to the relatively large average size of Neets Bay (579 mm) as compared to Gastineau (540 mm) and Hidden Falls fish (549 mm; Figure 1.2).

Variation in meristic counts was low (CVs = 0.02-0.09; n = 77-200). Pectoral fin ray counts differed among crosses in all comparisons that involved Neets Bay controls, which had the smallest mean fin ray count, although these differences were small (0.3-0.6 fin rays). Other differences between sexes and types of cross were weak ($0.01 < P < 0.05$), most often observed in gill raker counts, and of a small magnitude (0.1-0.4 fin rays or gill rakers).

When an effect of cross (parental, F_1, and F_2) was significant, the means of the character in hybrids were not consistently greater or less than parental means, and were sometimes intermediate but not necessarily equal to mid-parent values. The interaction of cross and sex significantly influenced length in one hybrid-cross ANOVA comparison (Gastineau-Hidden Falls F_1, $P < 0.001$; Appendix Table A7).

Fluctuating asymmetry

Branchiostegals exhibited significant DA in all three brood years ($P < 0.001$). In addition, gill rakers of the first lower arch exhibited significant DA only in BY97 returns ($P = 0.006$, Figure 1.3). We did not include branchiostegals or gill rakers of the first lower arch in the CFAs of BY97, and did not include branchiostegals in the CFAs of BY00.

Overall variances of hybrid and control CFA values did not differ within comparisons of either brood year after correcting for multiple tests, so we report results of *t*-tests of means using the homoscedastic formula. Mean CFAs of hybrids consistently exceeded those of parental controls in BY97, and CFA means of BY00 hybrids (both F_1 and F_2 types) were consistently lower than those of parental controls (Figure 1.4). Tests of differences from only one hybrid-parental comparison were significant: BY97 Gastineau – Neets Bay F_1 hybrids exhibited greater CFA1 values than Gastineau controls ($P = 0.03$). Analysis of the data after removal of 13 known inbred controls (half-siblings) from BY00 yielded similar results.

Variances of hybrid CFAs were greater than parental controls in BY97 in all but one comparison (GGNN-GGGG CFA5), while CFA variances of BY00 hybrids were generally smaller than parental controls, although none of these differences was significant (Figures 1.5 and 1.6). The interaction of cross and sex significantly influenced CFA index values in hybrid-cross ANOVA comparisons including Neets Bay controls and F_1 hybrids of Gastineau controls (CFA1 P = 0.007, CFA5 P = 0.02) and F_1 hybrids of Hidden Falls controls (CFA1 P = 0.03, Appendix Table A7).

Discussion

Survival

We did not observe evidence of outbreeding depression in this study. Survivals of F_2 hybrids did not differ from those of parental controls in BY00 returns. In contrast, survival of F_1 hybrids exceeded those of parental controls in BY97, but did not differ in the BY00 returns. One possible explanation for the increased survival of hybrids in the BY97 fish is heterosis.

Although we did not detect losses in fitness of outbred F_2 hybrids as compared to parental controls, the power of this test was very low (0.05). An F_2 hybrid survival of 0.5%, less than one-third of that observed, would be required for this test to achieve a power of 0.5 with our sample sizes; to achieve a power of 0.9, an F_2 hybrid survival of 0.1% would be required. These results are the same as those reported by Smoker et al.

(2004) but differ from other outbreeding depression studies of salmon populations. For F_2, but not F_1, hybrids between even- and odd-broodline pink salmon from Southeast Alaska, survival was lower (Gharrett et al. 1999). In another study of within-broodline hybridization of pink salmon, F_2 hybrids of spatially separated Alaskan populations in both even and odd brood lines and F_1 hybrids in one broodline had lower survival than parental controls (Gilk et al. 2004).

Trait variation

Length differed between sexes, among populations, and across brood years, which is not uncommon for coho salmon (Quinn 2005). The strong Population*Year significance observed in the stock-specific ANOVA indicates a genotype by environment effect, and the similar responses by populations to different brood years suggests plasticity. The significance of cross effects of sizes observed in the hybrid-cross models is attributable to the larger size of Neets Bay controls as compared to other controls and hybrids.

In contrast, we observed little variation in most of the meristic traits that we examined. Variation of several of the meristic traits across brood years probably reflects the different developmental and rearing environments experienced in those years. The traits that did not vary (i.e., fin rays) were the least variable traits, and are probably strongly canalized. The environments of different brood years represent a test of canalization against environmental perturbation. The second generation of hybridization, which was introduced in BY00, potentially disrupted epistatic interactions by altering the genetic background of hybrids, and provided a test of the strength of trait canalization against genetic perturbation.

The lack of significant differences between hybrids and controls (i.e., cross effect) for most traits suggested strong genetic canalization of these traits. In light of the small magnitude of differences in counts, and the absence of clear trends in trait variation, we interpreted the significant results that were observed as an artifact of the high leverage of non-modal observations. Similarly, although populations responded differently to brood years in three meristic traits, which suggested a genotype by environment interaction,

these result from a fraction of a count in the differences between means and may not be biologically meaningful.

Fluctuating asymmetry

The CFAs of outbred fish exceeded those of controls in only one instance, and they were often less than controls. There were no detectable differences between CFA means of BY00 parental controls and their hybrids. Variances of CFAs did not differ between parental controls and their hybrids in either brood year. Removal of known inbred individuals from parental control groups did not alter these results, which suggests that there is little variation in the genetic mechanisms of meristic trait canalization. Indeed, it is possible that heterosis contributed to canalization processes in BY00.

Conclusions

Previous reports documented differences in development time and embryo survival among these populations and their F_1 hybrids (Granath et al. 2004). However, we observed no evidence of outbreeding depression. The differences between the studies may have resulted from a number of factors: (1) previous work accurately described differences among these populations, but these differences were not great enough to engender hybrid losses in fitness as measured by marine survival or the fluctuating asymmetry of bilateral meristics after two generations of hybridization; (2) development time and embryo survival may not relate directly to marine survival and trait symmetry as was measured in our experiments; and/or (3) the power of our experiments was insufficient to detect differences.

Our objective was to compare attributes of three disparate sources of Southeast Alaskan coho salmon populations and determine if there were sufficient genetic differences among them to produce outbreeding depression. The work of Granath et al. demonstrated adaptive differences among these populations (2004). Coho salmon populations in Alaska have been characterized as small in abundance and influenced more by genetic drift than gene flow (Olsen et al. 2003). Olsen et al. observed greater intra-population genetic diversity in three Southeast Alaska coho salmon populations than

other Alaska coho salmon populations, and suggested that recent postglacial colonization from the southern refugium populations of British Columbia and the Pacific Northwest may have influenced their genetic compositions (2003). It is likely that recent colonization and gene flow has retarded divergence among Southeast Alaska coho salmon populations. We observed a lower F_{ST} of 0.028 ($P < 0.0001$) than that reported by Olsen et al. (0.049, 95% CI=0.02-0.083; 2003), but the microsatellite divergence among our source populations was strongly significant. Despite clear genetic divergence and the distinct geographies and selection forces that the three source populations experienced prior to culture (as evidenced by development rates in Granath et al. 2004), the differences were small enough that hybridization had little detectable effect on survival.

This study adds to a small but growing body of salmon research that investigates outbreeding depression into the second generation of hybridization. These results differ from an earlier study which reported an outbreeding depressive effect on marine survival (Gharrett et al. 1999) but not on the early development (Wang et al. 2006) of odd- and even-brood year pink salmon. Although we did not examine both marine survival and early development in the F_2 generation as was done in the pink salmon study, and genetic divergence between odd- and even-brood year pink salmon is undoubtedly greater than that among our three coho salmon populations, the difference in results of these two studies is further evidence that hybridization outcomes, at least in fish, are unpredictable (McClelland and Naish 2007). The results of this study should be interpreted with caution because other research has indicated that interbreeding of distinct salmon populations can result in losses in fitness (e.g., Gharrett et al. 1999; Gilk et al. 2004; McGinnity et al. 2003), and the differences among our experimental populations do not apply to other potential salmon hybridization conditions. Similarly, our study was conducted in one freshwater rearing and release environment (Gastineau); replicating this study at the other donor hatcheries would have allowed for a more robust test of genotype by environment interaction and for fitness losses under different environmental conditions. While the marked decrease in length frequencies through brood years may have been an artifact of ocean feeding conditions, it may be due in part to the effect of the Gastineau hatchery

environment and the decrease in relative fitness for nonlocal stocks that has been observed elsewhere (Araki et al. 2008). We were unable to incorporate freshwater spawning success in our tests of fitness; tests for fitness changes based upon relative reproductive success may have been more informative. For the long-term conservation of these biologically and socially important species, it is important to maintain genetic diversity and minimize the unnatural removal of barriers to gene flow. These goals can be advanced by maintaining healthy wild populations of Pacific salmon or, if hatcheries are used, choosing locally adapted broodstocks, minimizing the transplanting of stocks and potential for wild and hatchery fish to interbreed, or if that is not possible, minimizing the genetic and ecological divergence between hatchery and local wild fish by annually incorporating wild fish into hatchery broodstock.

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Tables

Table 1.1.—BY97 experimental design. Parental origins abbreviated Gastineau (G), Hidden Falls (H), and Neets Bay (N). Three parental lines in boldface and six F_1 hybrid lines in italics (female parent listed first) were produced from BY94 parents, released, and recovered in 2000.

		Sire		
		Gastineau	Hidden Falls	Neets Bay
Dam	Gastineau	**GG**	*GH*	*GN*
	Hidden Falls	*HG*	**HH**	*HN*
	Neets Bay	*NG*	*NH*	**NN**

Table 1.2.—BY00 experimental design. Parental origins abbreviated Gastineau (G), Hidden Falls (H), and Neets Bay (N). Three parental control lines in boldface, six replicate F_1 hybrid lines in italics, and six F_2 hybrid lines (female parent listed first). These crosses were made from returns of the BY97 crosses (Table 1), released, and recovered in 2003.

	Sire								
Dam	GG	GH	GN	HG	HH	HN	NG	NH	NN
GG	**GGGG**				*GGHH*				*GGNN*
GH				GHHG					
GN							GNNG		
HG		HGGH							
HH	*HHGG*				**HHHH**				*HHNN*
HN								HNNH	
NG			NGGN						
NH						NHHN			
NN	*NNGG*				*NNHH*				**NNNN**

Table 1.3.—Details of PCR amplification and references for microsatellite loci. All loci were amplified following: 1 cycle 95° C for 3 minutes; x cycles of 95° C 30 seconds, y° C 30 seconds, 72° C 45 seconds; 1 cycle 72° C for 7 minutes, where x is the number of cycles and y is the annealing temp.

Locus	Reference	Accession number	Annealing temperature (°C)	# of cycles
Ots 101	Small et al. 1998	N/A	50	35
Ots 208	Greig et al. 2003; Williamson et al. 2002	AF393187	55	30
Oki 1	Smith et al. 2001	AF055427	48	30
Oki 10	Smith et al. 2002	AF055435	50	30
Oki 16	Smith et al. 2003	AF055440	52	30
Oki 20	Smith et al. 2004	AF055444	56	25

Table 1.4.—Summary statistics of allele frequency analysis: probability of rejecting Hardy-Weinberg equilibrium (HWE) by population and globally, F-statistics, and the number of observed alleles (n_a) and the number of private alleles by population and the totals for all three populations.

	Gastineau	Hidden Falls	Neets Bay	Global
n	90	90	90	270
HWE	0.01	0.75	0.31	0.09
F_{IS}	0.004	-0.017	-0.027	-0.015
F_{ST}				0.028***
n_a				
Ots101	20	22	27	30
Oki1	10	9	10	11
Oki10	15	15	21	26
Oki16	16	10	14	21
Private alleles	5	6	14	

*** $P < 0.001$.

Table 1.5.—Pairwise F_{ST}'s of BY94 population comparisons.

	Gastineau	Hidden Falls
Hidden Falls	0.0315***	
Neets Bay	0.0263***	0.0262***

*** $P < 0.001$.

Table 1.6.—Proportion (Prop.) of releases (Rel.) that returned (Ret.) by broodyear, experimental group, and parental sources. Returned fish include recoveries at Sheep Creek and in the fisheries. Experimental groups and parental sources are abbreviated as in text. See Tables 1 and 2 for definition of parental sources.

		BY97			BY00		
Exp. group	Parental sources	Rel.	Ret.	Prop.	Rel.	Ret.	Prop.
P	GGGG	6,071	92	0.015	5,064	89	0.018
	HHHH	6,017	88	0.015	5,694	113	0.020
	NNNN	5,951	63	0.011	7,349	130	0.018
	Total parental	18,039	243	0.013	18,107	332	0.018
F_1	GGHH and HHGG	12,119	202	0.017	10,022	196	0.020
	GGNN and NNGG	12,035	189	0.016	11,750	220	0.019
	HHNN and NNHH	12,058	213	0.018	13,712	275	0.020
	Total F_1	36,212	604	0.017	35,484	691	0.019
F_2	GHHG and HGGH				14,028	252	0.018
	GNNG and NGGN				13,622	256	0.019
	HNNH and NHHN				15,019	247	0.016
	Total F_2				42,669	755	0.018
	Total hybrid	36,212	604	0.017	78,153	1,446	0.019
	Total	54,251	847	0.016	96,260	1,778	0.018

Table 1.7.—Test of homogeneity of survival among broodyears 1997 (BY97) and 2000 (BY00). *G* is the log-likelihood statistic, df is degrees of freedom, and *P* is the significance of the test.

Source of variation	BY97			BY00		
	G	df	*P*	*G*	df	*P*
Parents	5.78	2	0.06	1.02	2	0.60
Hybrids						
Among F_1 hybrids	1.39	2	0.50	0.58	2	0.75
Among F_2 hybrids				2.32	2	0.31
Total within hybrids				2.91	4	0.57
Between hybrids				3.31	1	0.07
Total hybrids	1.39	2	0.50	6.21	5	0.29
Between parents and hybrids	8.14	1	0.00	0.02	1	0.88
Total	15.31	5	0.01	7.26	8	0.51

Table 1.8.—Tests of effects of year, source population, and sex applied to the parental control populations of BY94, BY97 and BY00 for the stock-specific model for Kruskal-Wallis (Year and Population) and Mann-Whitney U (Sex) tests. Tests do not include corrections for multiple tests.

Character	Year	Population	Sex
Length (MEF)[a]	***	***	**
Pelvic rays (L)			
Pelvic rays (R)			
Pectoral rays (L)		**	
Pectoral rays (R)		*	*
Branchiostegals (L)	***		
Branchiostegals (R)	***		
Gill rakers			
1st arch, upper (L)	***		
1st arch, lower (L)	***	*	
1st arch, upper (R)	***		
1st arch, lower (R)	***	**	
2nd arch, upper (L)	***		
2nd arch, lower (L)	***		
2nd arch, upper (R)	***	*	
2nd arch, lower (R)	***		
CFA1	***		
CFA2	**		
CFA3	**		
CFA5	**		

[a]MEF is mid-eye to fork of tail; L and R are counts on the left and right sides, respectively.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 1.9.—Tests of sex and hybridization effects that compare two control populations and their pooled reciprocal hybrids (e.g., GGHH and HHGG F_1 crosses) in BY00 for the hybrid-cross model with Kruskal-Wallis (Cross) and Mann-Whitney U (Sex) tests. Results of tests of controls and F_1 hybrids precede slash (i.e. */), results of tests of controls and F_2 hybrids follow slash (i.e. /*); control populations are abbreviated as in text. Tests do not include corrections for multiple tests.

	GG-HH		GG-NN		HH-NN	
Character	Cross	Sex	Cross	Sex	Cross	Sex
Length (MEF)[a]	/*	/***	***/***	***/***	***/***	***/***
Pelvic rays (L)						
Pelvic rays (R)			*/			/**
Pectoral rays (L)			**/*		***/***	
Pectoral rays (R)	/*		**/**		***/***	
Branchiostegals (L)						
Branchiostegals (R)						
Gill rakers						
1st arch, upper (L)		*/				
1st arch, lower (L)	/*	*/		/*		
1st arch, upper (R)						
1st arch, lower (R)	/*					
2nd arch, upper (L)						
2nd arch, lower (L)						
2nd arch, upper (R)				*/		
2nd arch, lower (R)						
CFA1						
CFA2	*/					
CFA3						
CFA5						

[a]MEF is mid-eye to fork of tail; L and R are counts on the left and right sides, respectively.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Figures

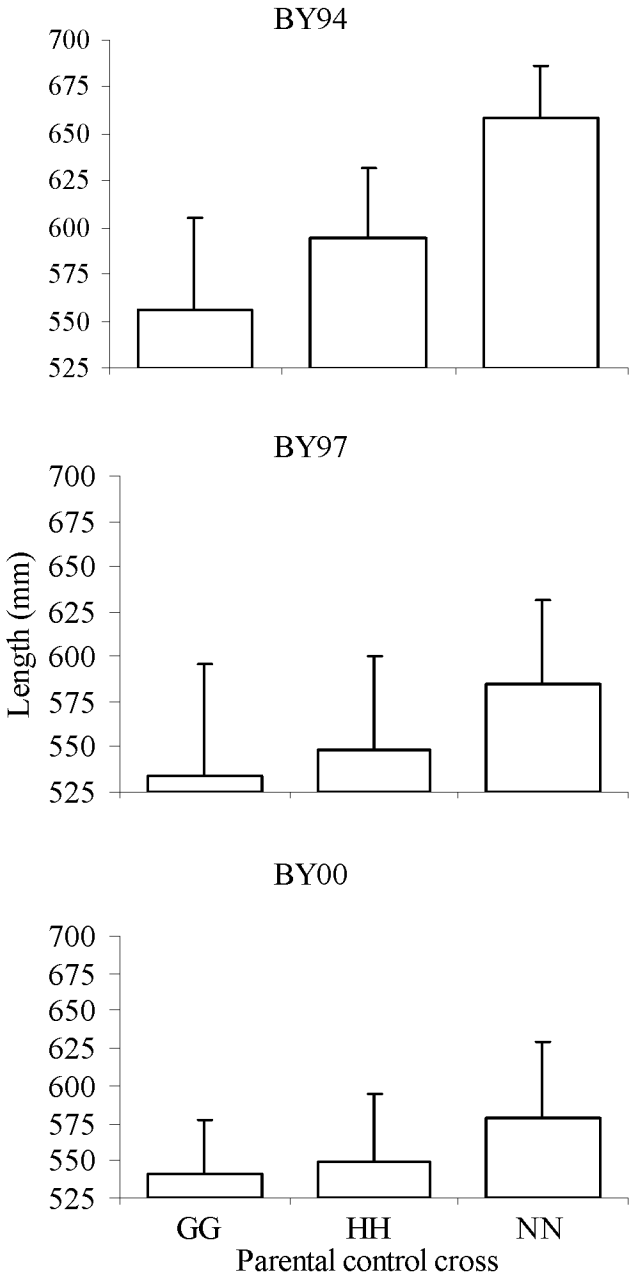

Figure 1.1.—Means and standard deviations of lengths of crosses in comparisons of parental controls (see Table 1) and brood years for the stock-specific model.

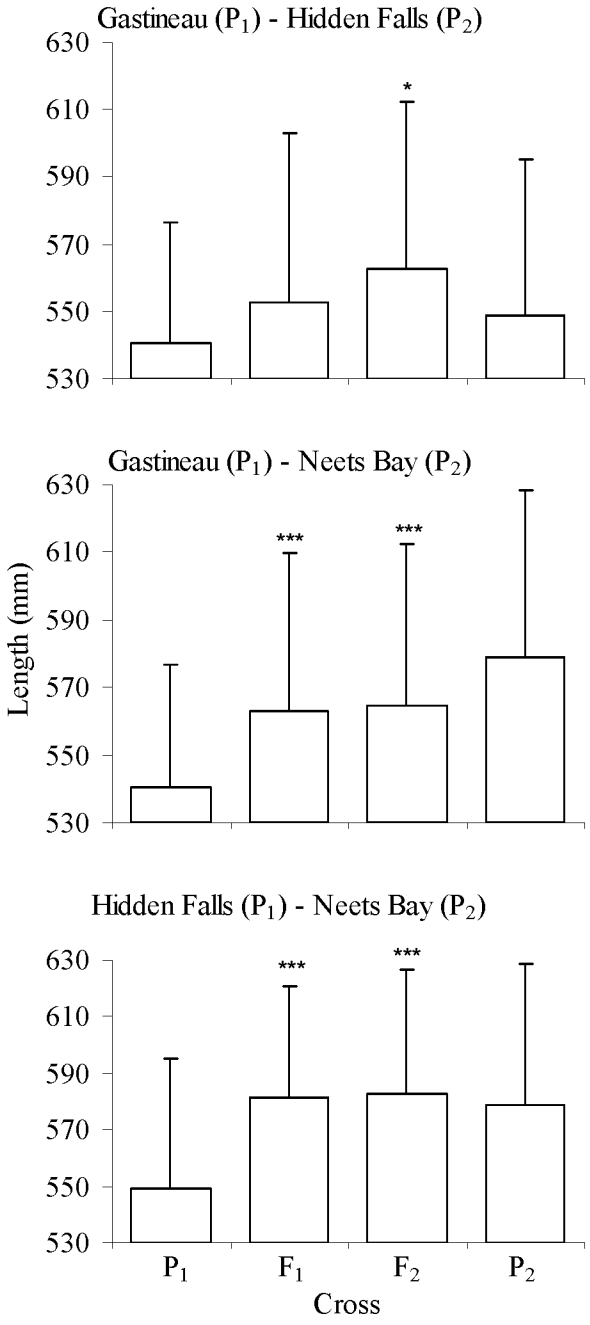

Figure 1.2.—Means and standard deviations of lengths of crosses in comparisons of hybrids and parental controls for the generation-specific models. Asterisks indicate significance of cross effect in comparisons including indicated hybrid and do not include corrections for multiple tests. * $P < 0.05$; *** $P < 0.001$.

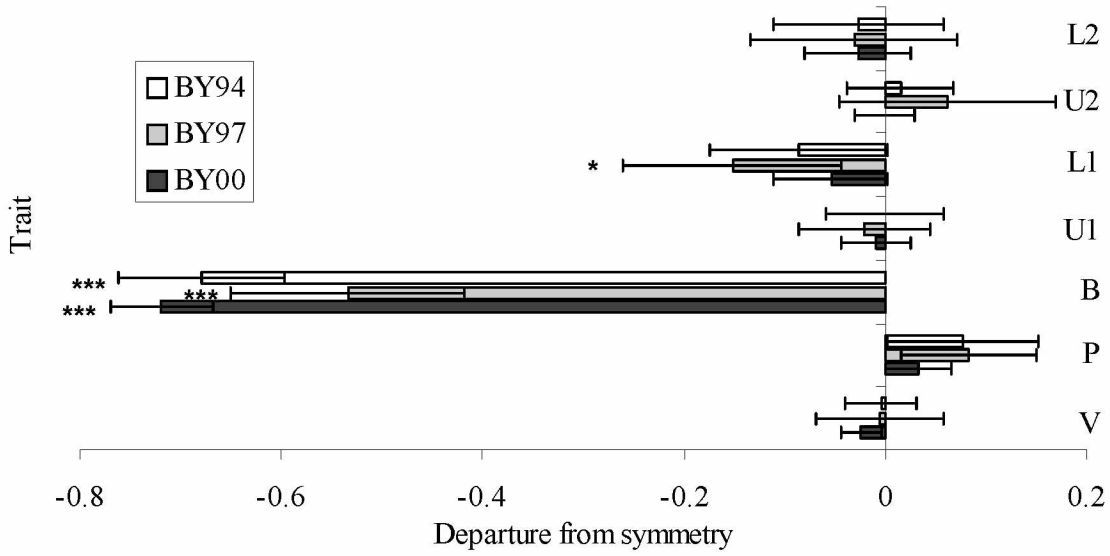

Figure 1.3.—(Right – Left) mean and 95% confidence interval for bilateral traits in parents from BY94, BY97 and BY00, and two-tailed *t*-test results. Traits abbreviated as in text. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; *t*-test results are corrected for seven multiple tests within brood year with a sequential Dunn-Sidak correction.

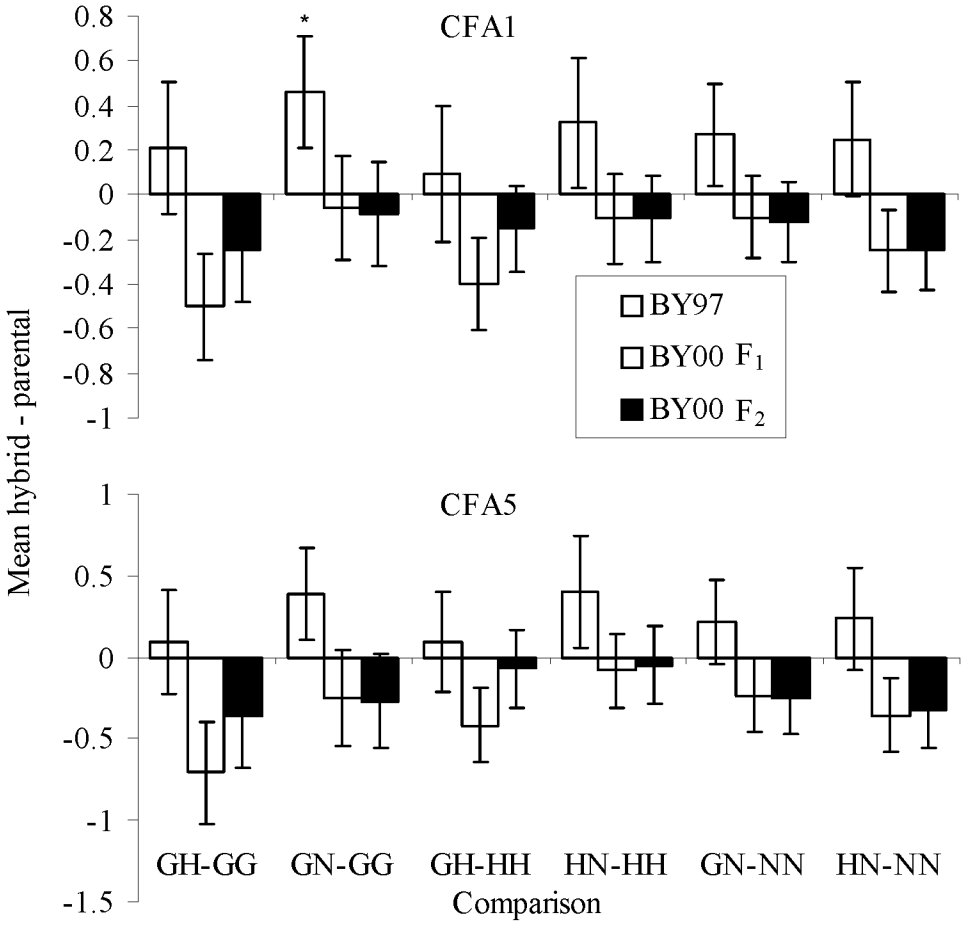

Figure 1.4.—Differences between CFA means of hybrids (F_1 in BY97, F_1 and F_2 in BY00) and parental controls in BY97 and BY00 ± standard error of the difference between means. Hybrid and parental controls are abbreviated as in text; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

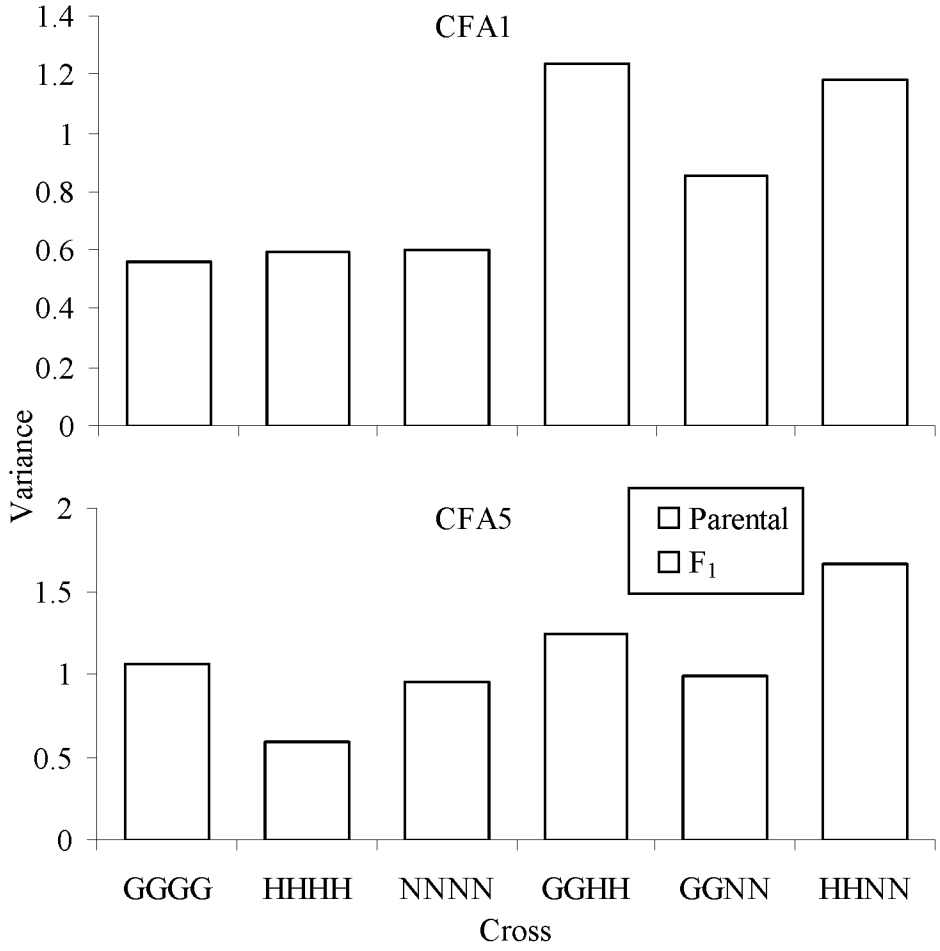

Figure 1.5.—Variances of CFA1 and CFA5 in BY97 crosses. Crosses abbreviated as in Table 2.

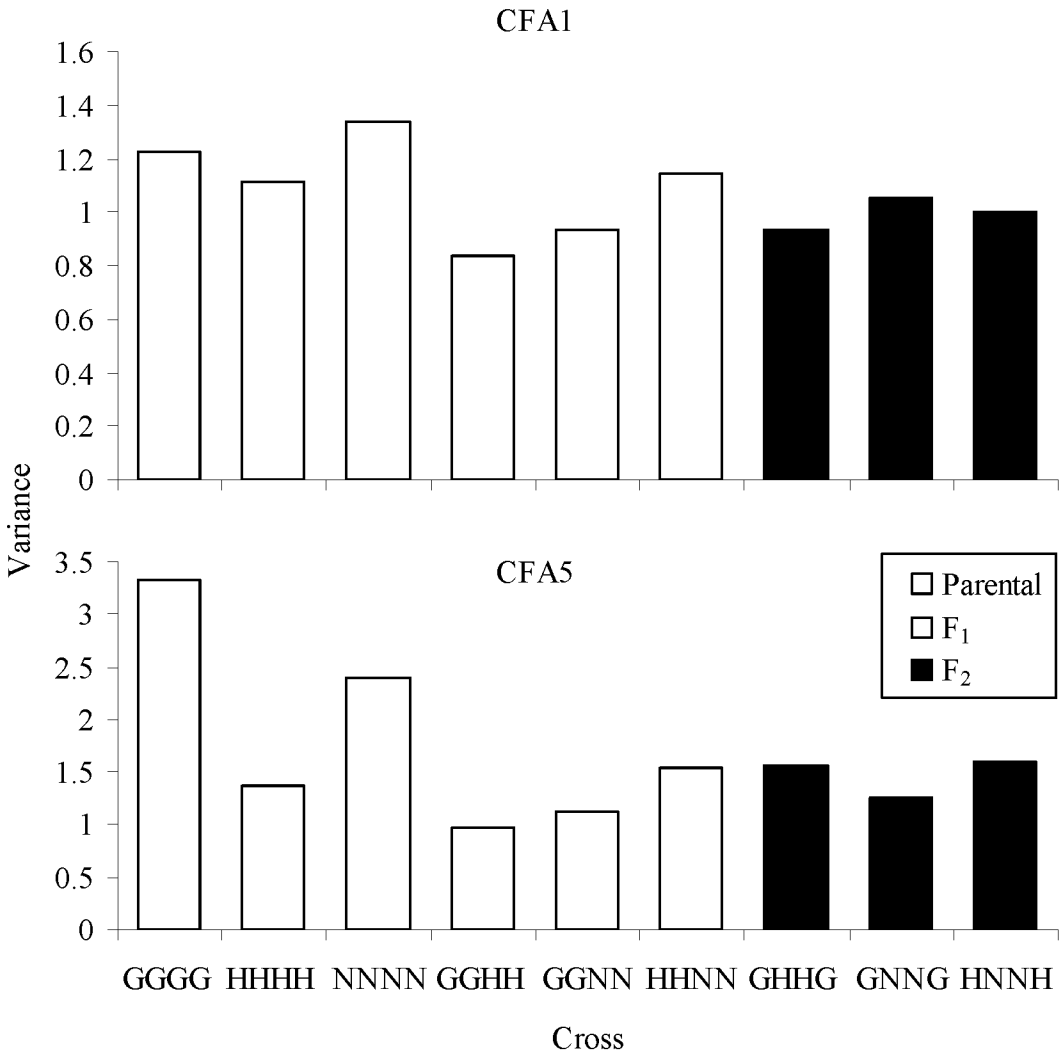

Figure 1.6.—Variances of CFA1 and CFA5 in BY00 crosses. Crosses abbreviated as in Table 2.

Chapter 2:

Quantitative genetic analysis of morphological and meristic traits in three populations of coho salmon and their hybrids[2]

Abstract

We investigated the quantitative genetics of length and bilateral meristic characters observed among three Southeast Alaska coho salmon (*Oncorhynchus kisutch*) populations hybridized in a study of outbreeding depression. The line cross analysis of length distributions suggested that both additive and dominance gene action affected gene expression contributing to population divergence; and two of the three analyses indicated that epistasis was influential in explaining trait distributions among the populations. In contrast, we failed to reject the additive gene action model for most of the meristic distributions. We observed no quantitative genetic variation for any of the characters in half- and full-sib analyses, but the power to detect these effects was low because the crosses included relatively few sires. Comparisons of population divergence as measured by quantitative traits (Q_{ST}) and neutral molecular markers (F_{ST}) suggested that divergent selection acts on length among these populations. Conversely, we observed little variability for meristic traits, which suggests that either there is little variation on which selection can act or selection is convergent. These results are consistent with the idea that length may be an adaptive trait in the three coho salmon populations included in this study, but that bilateral meristic traits, which show little variation within or among populations, are highly conserved characters.

[2] Dann, T.H., W.W. Smoker, J.J. Hard, and A.J. Gharrett. 2009. Quantitative genetic analysis of morphological and meristic traits in three populations of coho salmon and their hybrids. Prepared for submission to the Transactions of the American Fisheries Society.

Introduction

Distinct populations often differ genotypically as a response to diversifying local environmental conditions or random drift as a result of reproductive isolation. Phenotypic variation within different populations may reflect the influences of different natural selection regimes, a result that is often referred to as local adaptation. The process of local adaptation is of interest because it plays a consequential role in promoting genetic divergence between populations, which is important for the evolutionary potential and long term viability of species. Interbreeding between distinct populations can erode local adaptation and result in fitness loss, which is known as outbreeding depression.

Analysis of genetic diversity is an important tool for the sustainable management of fishery resources and for aquaculture. Pacific salmon (*Oncorhynchus spp.*) have received considerable attention for both the selection of traits desirable in culture (e.g., harvest weight of coho salmon *Oncorhynchus kisutch*; Neira et al. 2006) and an understanding of their evolutionary history and adaptive capabilities in the face of anthropogenic and climate changes (reviewed in Carlson and Seamons 2008). Understanding the mechanisms of inheritance of quantitative traits, particularly those that underlie locally adapted phenotypes, can help elucidate the evolutionary history of a species and mitigate against losses of the adaptive variation that may be important to genetic fitness and can guide programs of broodstock management in aquaculture.

We previously observed substantial phenotypic differences among three Southeast Alaska coho salmon populations that were hybridized in a study of outbreeding depression (Chapter 1). Significant genetic variation of development rate and differences in embryo survival among these populations has also been observed (Granath et al. 2004). In studies of pink salmon (*Oncorhynchus gorbuscha*), second generation and some first-generation hybrids between different populations exhibited reduced survival compared to fish derived solely from the parental populations that suggested outbreeding depression (Gilk et al. 2004). Those results were interpreted as a consequence of disruption of coadapted epistatic interactions among loci, which had developed in the process of local adaptation as a result of different local environments coupled with

random genetic drift. In contrast, no significant reductions in survival were detected in first- and second-generation hybrids between the coho salmon populations observed in this experiment as compared to salmon derived solely from the populations. No losses of fitness, as estimated from differences in embryonic survival and marine survival of control and hybrid groups, were detected, but the power of those tests was low (Smoker et al. 2004). We also did not detect signs of outbreeding depression in tests for increases in the fluctuating asymmetry of bilateral meristics in hybrids, which would be an indication of reduced developmental stability (Chapter 1).

We investigated the genetic variation of adaptive traits among the three coho salmon populations included in this study. In particular we examined length at maturity and meristic traits that are distributed bilaterally on the body. Size is a trait that has been observed to respond to local physical environmental differences (Taylor and McPhail 1985) and to be important in local adaptation of Pacific salmon populations. In addition, size may respond to interannual variation in freshwater spawning habitat (Carlson and Quinn 2007) and marine environments (Farley et al. 2007) that is commonly encountered by most Pacific salmon. Size plays an important role in sexual selection in some species (e.g., Quinn and Foote 1994). Variation in size either reflects genetic variation, phenotypic plasticity, or both. In contrast, bilaterally measured meristic traits indicate whether individuals maintain a locally adaptive shape and are probably highly canalized, producing consistent and conservative conformations in species. We analyzed variations of size and of meristic traits separately because it is likely that the two kinds of traits differ in their evolutionary history of adaptation and influence on fitness.

We used two approaches to examine and partition the quantitative genetic variation of those traits. Our primary focus was a model-fitting procedure referred to as line cross analysis (Lynch and Walsh 1998). We also analyzed variation among full- and half-sibling crosses, which is the classical approach to quantitative genetics, to partition the phenotypic variation observed in populations into environmental and genetic components.

Line cross analysis involves crossing individuals of different populations, parental population controls (P_1 and P_2), and their first (F_1) and second (F_2) generation hybrids as well as various intercrosses with the parental sources, to create a set of experimental groups of known pedigree. Individuals from these experimental groups are measured for quantitative characters of interest. Based on the known genetic background of each group, models that incorporate different modes of gene action (e.g., additive, dominance, and epistatic) are fitted to the means and variances of the observed phenotypes. Line cross analysis has revealed complex genetic architecture in populations of beetles (Demuth and Wade 2007), estimated the genetic effects on length, weight, and growth rate in strains of coho salmon (*Oncorhynchus kisutch*; Tymchuk et al. 2006; McClelland et al. 2005), and found widespread nonadditive effects on life-history and morphological traits of salmon (Roff and Emerson 2006).

The more often used approach for estimating heritabilities of phenotypic characters is based on the covariance of full- and half-sibling character data. Many aspects of salmonid life history and phenotype are heritable, such as development rate (e.g., pink salmon *Oncorhynchus gorbuscha*; Hebert et al. 1998), mortality caused by natural perturbations in the marine environment (e.g., Chinook salmon *Oncorhynchus tshawytscha*; Hard et al. 2000), and day of entry on spawning grounds (e.g., pink salmon *Oncorhynchus gorbuscha*; Smoker et al. 1998; Dickerson et al. 2005). If a quantitative trait diverges among populations because it has responded to local environmental differences, the distribution of the quantitative genetic variation among populations should also reflect that divergence.

The fixation index F_{ST} is often used to partition variation observed in molecular genetic markers among populations. The fixation index is a ratio of the variance in allele frequency among populations to the overall variance of allele frequencies in the entire set of populations. When measured from neutral loci, F_{ST} estimates the divergence among populations that results at an equilibrium between gene flow and random drift. The variation in quantitative traits can be partitioned similarly to estimate a statistic called Q_{ST} (Spitze 1993). The migration-drift equilibrium at loci that contribute to quantitative traits

is expected to produce estimates of Q_{ST} that are similar to those of neutral loci underlying F_{ST} estimates. However, if local adaptation (diversifying natural selection) also influences the allele frequency distribution among populations, Q_{ST} estimates are expected to exceed F_{ST} estimates for those populations (Spitze 1993). Convergent (purifying) selection would produce lower estimates of Q_{ST} than F_{ST}. This approach has revealed diversifying selection between two populations of coho salmon for juvenile growth rates (McClelland and Naish 2007) and between anadromous and resident brook charr for quantitative traits measured early in development (Perry et al. 2005).

Here we analyze the inheritance of length and of a suite of bilateral meristic traits that may contribute to local adaptation. Specifically we estimate: (1) the extent to which the traits are heritable; (2) which of the models that incorporates additive, additive and dominance, or epistatic gene action best explains the way in which the traits differ genetically among the populations; and (3) whether local adaptation may have played a role in determining the phenotypes of the traits in these populations. From these analyses, we evaluate the potential for combining line cross analysis and classical half-sib family analysis in a single experiment.

Methods

Field methods

We crossed coho salmon from Neets Bay, Hidden Falls, and Gastineau (also known as Macaulay or DIPAC) hatchery stocks, which had been separately derived from natural populations living in three geographically different southeast Alaska river basins. The Neets Bay lineage was established in 1982 and originated from Indian Creek, a tributary of the Chickamin River, near Ketchikan, Alaska, in Misty Fiords National Monument. The Indian Creek stock is the southernmost population in the study and is fed by high mountain streams. The Hidden Falls population was established in 1985 and originated from an unnamed, lake-fed stream that enters Deep Cove on southern Baranof Island, a low-altitude short drainage. The Gastineau population was also established in 1985; it originated from Montana Creek, an intermediate altitude tributary of the

Mendenhall River, which is near Juneau, Alaska and is the northernmost population in the study. These populations had been artificially cultured for three or four generations at the onset of the study, and serve as a proxy for the wild populations from which they were derived. It was not possible to compare hybrids in each parental environment, but all experimental crosses experienced the same rearing conditions. The standardized rearing conditions employed technology similar to that used at each of the donating hatcheries. This included Heath (Flex A Lite ™) incubators, manufactured semi-moist diets at recommended rations, and the release of yearling smolts to the estuary in the spring.

We flew gametes from Hidden Falls and Neets Bay hatcheries to Gastineau Hatchery on 6 November 1997 and spawned them on the same date in a full-sibling design to create brood year 1997 (BY97; Granath et al. 2004). We used gametes from 50 males and 50 females from each hatchery as the initial broodstock, but after removing individuals that tested positive for bacterial kidney disease (*Renibacterium salmoninarum*), between 32 and 45 males and 32 and 45 females from each hatchery were included as broodstock. We created nine different crosses in the F_1 generation: three sets of parental controls, one for each parental source, and six reciprocal F_1 hybrid crosses between the parental sources (Table 2.1 and Appendix Table A1). We reared the crosses in similar incubation and raceway environments at the Sheep Creek hatchery facility near Juneau (described in Granath et al. 2004), released them to sea (N = 54,251), and captured them as returning adults at Sheep Creek (N = 156). On 16 November 2000 we crossed the mature BY97 adults in a half-sibling design to produce the 15 F_2 experimental groups of brood year 2000 (BY00): three sets of controls (the parental lines), six reciprocal F_1 crosses, and six reciprocal F_2 hybrid crosses (Table 2.2 and Appendix Table A2). We reared these crosses in incubation and raceway environments that were similar to those of the first generation, released them to sea, and captured them as returning adults (N = 1,026). We implanted coded-wire tags in the snouts of smolts before they were released. The codes on the tags denoted the origin of the stock(s)

(Gastineau, Hidden Falls, and Neets Bay) and the type of cross (parental control or F_1 hybrid) of the parents of each smolt.

We collected heart tissue from returning BY00 fish for DNA analysis and froze fish for future morphological analysis. We preserved heart tissue (Seutin et al. 1991) and stored it at -20°C until DNA isolation. We obtained sex, mid-eye fork length (MEFL), and meristic counts from thawed fish. We counted pectoral (P) and pelvic (V) fin rays, branchiostegals (B), and both upper and lower (U and L) gill rakers on the first and second (1 and 2) gill arches. We separated fin rays with scalpels for counting and removed entire gill arches from fish for gill raker counts. We included the middle gill raker on each arch with the count of the lower gill rakers. We took two independent counts on each trait for quality control and resolved the few discrepancies by discussion between observers and additional counting until a consensus was reached. Although this approach precluded estimates of measurement error, it produced very accurate data.

Laboratory methods

We isolated total genomic DNA through the Puregene® DNA purification protocol for fish tissue (Gentra Systems, Valencia, CA). We amplified microsatellite loci by PCR in a Stratagene (La Jolla, CA) 96 Robocycler™. The reaction mixtures were 10 μL volumes that included approximately 1 unit of *Taq* polymerase and final concentrations of: 1X PCR buffer (50 mM KCl, 10 mM Tris HCl pH 8.3), 0.25 mM $MgCl_2$, 0.125 mM of each deoxynucleotide triphosphate (dNTP), approximately 0.05 to 0.10 μg DNA template, and 0.35, 0.4, and 0.04 μM of forward, reverse, and labeled primer, respectively, overlaid with mineral oil. The labeled primer was one of two IRDye® infrared dyes (LI-COR, Lincoln, Nebraska), which fluoresce at either 700 or 800 nm to visualize the PCR products. We used six loci in this study: Oki1, Oki10, Oki16, and Oki20 (Smith et al. 1998); Ots101 (Small et al. 1998); and Ots208 (also known as OtsG68; Greig et al. 2003, Williamson et al. 2002, respectively). The PCR conditions, locus information, and microsatellite analysis were described in Chapter 1.

Statistical methods

Parentage.—We used microsatellite genotypes to assign maturing offspring to parental pairs by exclusion analysis based on known parent matings (PROBMAX Version 1.3; Danzmann 1997). We determined types of crosses from assigned parent pairs and verified them by CWT (present in 96% of the fish).

Line cross analysis.—We used the joint-scaling test (Hard et al. 1992; Lynch and Walsh 1998) to evaluate the fit of the lengths and meristic observations of adults to additive and additive plus dominance models in the crosses made in BY00. Briefly, we quantified the means and variances of characters in the two groups of parental controls and their F_1 and F_2 hybrid crosses. We fit these data to models that evaluate the relative contribution of additive (A) and additive plus dominance (A-D) gene action to character means in these different groups. The additive model was the null model. Failure to reject that model can be consistent with very low divergence among lines for the trait(s) tested. We used the likelihood-ratio test statistic described in Lynch and Walsh (1998) to test whether the fit of the model was improved by including a dominance (D) term. Because we did not have a sufficient number of lines to test the fit of the data to epistatic models, we used the delta ratio *t*-test method to detect epistatic effects (E; Lynch and Walsh 1998).

Heritability analysis.—We used the covariance among sibling to estimate heritabilities for characters in each control population. We used subsets of the data from the returns of the BY97 and BY00 crosses to estimate heritabilities of length and meristic characters; the subsets included all families for which data from multiple offspring were available. Where possible, we used PROC MIXED (SAS version 9.1; SAS Institute 2004) to estimate the significance of covariance terms in both data sets. This maximum likelihood procedure is an improved alternative to traditional analysis of variance (ANOVA) variance component estimation because unbalanced designs do not bias variance component estimates as severely as does the generalized linear model (GLM) approach, and it simultaneously uses all of the available data and accounts for any nonindependence (Lynch and Walsh 1998). We used the REML (i.e., restricted

maximum likelihood) method of PROC MIXED, which, unlike the ML estimator, does not assume that all fixed effects are known without error and maximizes only the portion of the likelihood that does not depend on the fixed effects. Some of the PROC MIXED analyses failed to converge on parameter estimates. We replaced those failed estimates with analyses that used PROC GLM (SAS Institute Inc. 2004) to estimate the significance of covariance terms. We treated dam and sire effects as random effects and population as a fixed effect in both procedures.

We created all BY97 families with full-sibling matings (i.e., single dam per sire), so the model used to estimate heritability for each character was:

$Y_{ijk} = \mu + population_i + dam_{ij} + e_{ijk}$

where Y_{ijk} is the observed character of the k^{th} offspring of the j^{th} family, in population i; $population_i$ is the effect of the i^{th} population; dam_{ij} is the effect of the j^{th} family of the i^{th} population; and e_{ijk} is the residual error. In this model the covariance among full siblings estimates half the additive genetic variance, as well as one quarter of the dominance variance, the common (maternal) environment effects, and other epistatic terms. The term $population_i$ was removed in tests of individual populations.

We created the BY00 families with half-sibling matings (i.e., multiple dams per sire), so the model used to estimate heritability for each character was:

$Y_{ijkl} = \mu + population_i + sire_{ij} + dam_{ijk} + e_{ijkl}$

where Y_{ijkl} is the observed character of the l^{th} offspring of the j^{th} sire and k^{th} dam, in population i; $population_i$ is the effect of the i^{th} population; $sire_{ij}$ is the sire effect of the j^{th} father of the i^{th} population; dam_{ijk} is the dam effect of the k^{th} mother mated to the j^{th} sire of the i^{th} population; and e_{ijkl} is the residual error. In this model, the covariance among offspring of a sire estimates one-quarter of the additive variance and fractions of some of the epistatic sources of variance; the covariance among offspring of a dam estimates one-quarter of the additive variance, one-quarter of the dominance variance, the environmental variance that is due to a common maternal environment, and fractions of the epistatic sources of variance. The term $population_i$ was removed in tests of individual populations.

Q_{ST} analysis.—We compared a measure of genetic divergence among populations that was estimated for quantitative traits (Q_{ST}) to a measure that was estimated for neutral loci (F_{ST}) to investigate the possible effects of selection acting on the quantitative traits. We analyzed Q_{ST} for length and combined the results of meristic measurements into a single composite Q_{ST} value because it is likely that selective forces affect size and shape differently. Analyzing length individually and meristic characters collectively asks whether length is a locally adapted trait and whether meristic characters on average adapt to local conditions, respectively.

A Q_{ST} greater than F_{ST} indicates divergent selection for a trait whereas the opposite indicates either convergent selection or very low additive variation, when F_{ST}*'s* are estimated from neutral markers (Spitze, 1993). The value Q_{ST} was calculated for BY00 data following Spitze (1993) and O'Hara and Merilä (2005):

$Q_{ST} = V_{population} / (V_{population} + 2V_A) = V_{population} / (V_{population} + 8V_{sire})$.

The ANOVA model from which variance estimates were taken was:

$Y_{ijkl} = \mu + population_i + sire_{ij} + dam_{ijk} + e_{ijkl}$; all effects were considered random effects. The additive variance (V_A) for a trait was estimated as four times the sire variance estimated from this model. A composite Q_{ST} for meristics was calculated in a manner suggested for computation of composite F_{ST} (θ) for multiple loci (Weir and Cockerham 1984):

$$Q_{ST}(composite) = \sum_{trait} V_{population_i} / \left(\sum_{trait} \left[V_{population_i} + 8V_{sire_i} \right] \right);$$

each summation included only those variance components from ANOVA models that successfully converged.

Q_{ST} was compared to F_{ST} to assess the composite form of selection affecting variation among the populations in our observed traits. We found no evidence for selection acting upon our microsatellite loci after evaluating our data set with the workbench LOSITAN (Beaumont and Nichols 1996; Antao et al. 2008). The length and composite meristic Q_{ST} estimates were compared to an F_{ST} distribution that was obtained from the following bootstrap routine: alleles were randomly sampled from the set of all

alleles at all loci in the original BY94 data set to reconstruct the total number of alleles in the genotypes observed in the original data set, irrespective of their loci. We used ANOVA to compute θ for each bootstrap iteration as described by Weir and Cockerham (1984). Note that the estimate of θ is computed from separate analyses of each allele. The 95% confidence interval of this distribution was bounded by the 250^{th} and 9750^{th} estimates of the ranked 10,000 iterations. We also examined the variance of F_{ST} among loci with the Lewontin-Krakauer test described by Whitlock (2008). We used BY94 allele frequencies because they provide the best estimates of the divergence among these populations. Although standard tests for significance were not possible without a measure of error for our Q_{ST} estimates, this method allowed a qualitative comparison with which to assess the form of selection acting upon length and meristics.

Results

Parentage

We captured and genotyped 943 BY00 fish that we were able to assign to BY97 parents. Length measurements were taken from 698 adult salmon returning to Sheep Creek from BY00 crosses and 281 BY97 Sheep Creek returns; counts of bilateral traits were made for 644 BY00 and 281 BY97 Sheep Creek returns. The number of individuals with character data in the three line crosses (i.e., Gastineau-Hidden Falls, Gastineau-Neets Bay, and Hidden Falls-Neets Bay) ranged from 209 to 316 for length and 210 to 304 for bilateral traits (Table 2.3).

Line cross analysis

Length.— Each line cross analysis produced different results (Table 2.4 and Figure 2.1). Length variation in the Gastineau-Neets Bay analysis was best explained by a simple additive model, a dominance term did not improve the fit, and there was no evidence of epistasis. Inclusion of a dominance term in addition to the additive term significantly improved the fit of the model ($P = 0.01$) for the Hidden Falls-Neets Bay analysis, and a test for the influence of epistasis was also significant ($P < 0.001$). An additive model best explained the variation among means of line crosses in the

Gastineau-Hidden Falls analysis, but the fit to the data was poor ($P = 0.06$) However, the test for epistasis was significant ($P < 0.001$).

Bilateral traits.—An additive model best explained the mechanism of inheritance of most of the bilateral traits that we examined (Table 2.4). There was no variation in pelvic fin rays for two of the three line crosses (Gastineau-Neets Bay and Hidden Falls-Neets Bay), so those analyses were not possible, and six of the analyses were inconclusive because none of the models tested fit the data well. In only one case did the additive plus dominance model provide the best fit to the data (Gastineau-Hidden Falls LR1), although the fit to the model was weak ($P = 0.06$). This was the only instance in which the additive plus dominance model fit the data best; however, inclusion of the dominance parameter did significantly improve the fit of the model in three of 42 bilateral trait analyses. In two of these cases (Gastineau-Hidden Falls PR, Gastineau-Neets Bay VR) the improvement was slight, and in the third (Gastineau-Hidden Falls UL1) the fit of the model improved, although an additive plus dominance model still did not fit the data adequately (Appendix Table A8).

Heritability analysis

The number of offspring used in the heritability analyses was sometimes low (n = 29 to 127), especially for BY97, because those families often had fewer than two returning offspring and character data were not obtained from all returning fish (Table 2.5). More important for detecting dam or sire effects, the number of sires was often low (n = 13 to 15).

Length.—Genetic covariance parameters did not differ significantly from zero for length in any population in either brood year but did differ between populations in both brood years (Table 2.6). The differences among populations are similar to length differences previously observed in variance partitioning models (Chapter 1). In analyses within each population, length had no significant effect (Table 2.7).

Bilateral traits.—Genetic covariance parameters did not differ significantly from zero for most characters in either brood year (Table 2.6). When the likelihood procedure successfully converged on parameter estimates, only one was significant (BY97 UR1

Dam $P = 0.048$). One trait lacked variation from which to evaluate BY97 dam significance (VR). In the five analyses in which the likelihood procedure failed to converge on BY00 parameter estimates, GLM analyses indicated significance of dam effects but not of sire effects. The GLM analyses of dam effect were significant in four of the five analyses (LL1, LR1, UL2, and LR2) and highly significant for one (UL1). When the effect of population was significant, differences among populations were similar to that detected by variance partitioning models (Chapter 1). In analyses of individual source populations, some meristic counts were influenced by dam effects, particularly in the Neets Bay stock. Sire effects were not significant except for UL2 in the Hidden Falls stock (Table 2.7).

Q_{ST} analysis

The Q_{ST} estimate for length exceeded both the estimate of F_{ST} and its upper bound of the 95% confidence interval estimated by both the bootstrapping and Lewontin-Krakauer methods (Table 2.8, Figure 2.2). The composite Q_{ST} estimate for meristics exceeded the estimate of F_{ST} but was within both 95% confidence intervals. One meristic character (LR1) had a population variance component that was much higher than that of all other meristics. This variability in gill raker counts is similar to differences among populations that were previously observed in variance partitioning models; these differences are small and may not be biologically meaningful (Chapter 1). So, we also calculated a composite Q_{ST} for meristics that did not include LR1 and compared this estimate to F_{ST}. This composite Q_{ST} estimate was less than the estimate of F_{ST} and the lower bound of the bootstrap confidence interval but fell within the Lewontin-Krakauer interval, which was based on just one degree of freedom.

Discussion

We analyzed the distributions of traits among three populations and their hybrids to determine which traits were heritable, to identify which model of gene action best explained distributions of the traits, and to determine if local selection differences may have played roles in determining the phenotypes of the traits in these populations.

We evaluated two types of traits, length and several meristic characters. Length is likely to be influenced by differences in life history experiences such as nutrition, whereas meristic bilateral characteristics underlie the morphology of each individual and are completed early in development. The differences between the results for length and bilateral meristic traits in these coho salmon populations are pronounced and warrant a short review to provide a context for our analyses. Expression of length as a trait culminates at maturity. Length accrues during the salmon's entire life and is influenced by a sequence of different environments. Beginning with the maternal effect that egg size and yolk availability have on growth (Einum and Fleming 1999), the water temperature of the stream habitat in which alevin develop greatly influences the rate of yolk absorption and growth opportunity during this early stage of life (Heming 1982). Habitat availability and primary production of rearing streams affect the growth and length of rearing juveniles prior to ocean migration (reviewed in Quinn 2005). The estuarine habitat presents a productive foraging environment for salmonid growth given favorable conditions (Thorpe 1994). However, adult salmonid length is most dependent upon ocean conditions, and most of the length observed at maturity accrues during the final few months of marine life (reviewed in Quinn 2005). Interannual variation in any of these environments can affect the length achieved at maturity, and salmonids have evolved many adaptations to succeed in these differing environments. Another consideration is that length may play a role in both natural and sexual selection of salmonids (Hamon and Foote 2005) and, consequently, be influenced by these forces.

Genetic factors that influence length have been documented at several life history stages of salmon, notably the effect of growth hormone at different stages (Bjornsson 1997). We might also expect that length would be influenced by epistasis, which we observed in two of the line crosses, as a result of the diversity of environmental and life stage influences on length that interact with one another, the genetic factors likely associated with these influences, and the sex-linkage for genetic factors associated with length observed in other salmonids (Forbes et al. 1994; Perry et al. 2003). Length at

maturity can also be influenced by interactions between the genotype and environment (e.g., rainbow trout *Oncorhynchus mykiss*; McKay et al. 1984).

In contrast to expression of length, the number of elements for meristic counts has little variation; the number is generally fixed early in development – by hatching for some characters, and the number remains stable during the individual's life (Beacham 1990; Beacham and Murray 1986). Several of these traits are distributed bilaterally on the fish, and presumably the structures have evolved to maintain a consistent symmetric form. Although the development process for these meristic traits probably involves many loci and epistatic interactions, the expression differs from that of length in that the meristic traits exhibit little variability and are probably strongly canalized.
The evolutionary history of meristic traits probably involved strong stabilizing selection. The resulting system now involves coordination of the loci involved to produce a developmental process that repeatably produces the conservative form, even in the presence of environmental variation during early development. Indeed, tests of canalization to environmental and genetic perturbation indicated strong canalization for these traits and that the variability observed reflects phenotypic plasticity (Chapter 1).

Length variation was best explained by an additive model in two of the line cross analyses and by an additive plus dominance model in the third, and one analysis from each result showed strong indications of epistatic effects. Although the delta ratio *t*-test for epistasis that we used is not as rigorous as the joint scaling test for additive and dominance gene action, it still provided evidence for an epistatic influence on length distributions in two of three line cross analyses. In a previous study of coho salmon, length variation among crosses of two coho populations was adequately explained by an additive plus dominance model. However, that study carried too few lines to apply the joint-scaling test for an epistatic model and did not report results of the delta ratio *t*-test (McClelland et al. 2005).

In contrast to length, the majority of bilateral character distributions were best explained by an additive model, and models that included dominance fit the data poorly. A few of the analyses were inconclusive because characters were not explained well by

any model, which suggests the possibility that more complex models might better explain their distributions. Although the consistency with which the additive model explained results among line crosses and characters would seem to support an additive model for bilateral trait distribution, it is important to recognize that the ability of the joint scaling test to resolve additive versus other effects in contributing to divergence depends on a certain amount of divergence between parental lines, and their differences from corresponding hybrid lines. The joint scaling test assumes normally distributed, quantitative traits—characteristics not satisfied by the bilateral traits we examined. Furthermore, when variation in a trait is very low, the ability of joint scaling tests to reliably identify genetic mechanisms of divergence is probably questionable. The low variability observed within and among populations (Chapter 1) is consistent with an evolutionary history that has resulted in highly conserved characters, which are evidently difficult for the joint scaling test to evaluate. In the case of bilateral meristic characters, the support for any particular genetic model of divergence is probably best interpreted simply as evidence of significant population differentiation, as might be found with a simple nonparametric comparison (the joint scaling test is related to the chi square test).

The significance of many of the line cross analyses suggests that significant sire and/or dam effects in heritability analyses might be detected with sufficient power. Line cross analyses measure how well a model incorporating additive and in some instances dominance gene action fits observed trait distributions, whereas the heritability analysis estimates some of the additive and dominance variance with the portion of the variance that is attributable to additive or non-additive variation dependent on the mating design. We did not observe significant effects for length or meristics when the likelihood procedure successfully converged on parameter estimates and, due to the unbalanced nature of our data, GLM results must be interpreted cautiously. Length exhibited relatively high heritabilities in a population of brown trout, but estimates of heritability differed among periods of the juvenile life stage (Blanc 2005). Our single measurement of length occurred at maturity; the absence of significant covariance parameters for heritability may reflect the inadequacy of our experimental design to describe genetic

variance that results from growth during different stages of life. It may also be that our covariance parameters are imprecise because both dam (both broods) and sire (BY00) effects incorporate some epistatic sources of variance, especially considering the line cross analysis results. More likely, the lack of significant heritability estimates is due to small sample sizes, particularly the small number of sires involved in our mating designs (Table 2.5).

Line cross and heritability analyses present different approaches to investigating quantitative genetic variation in these traits. Line cross analysis evaluates the composite mode of gene action contributing to divergence among populations, whereas heritability estimation generally focuses on within-population variation and does not concern the type of gene action but whether a genetic component significantly influences the variance observed for a character. The primary focus of our experimental design was the line crosses. Consequently, the number of half-sib families was limited. A design that includes both line crosses and half-sib crosses must take care to produce a sufficient number of crosses to provide the statistical power to resolve sire effects. The line cross analysis complemented another aspect of our study: it offered an approach to test for the potential strength of intrinsic outbreeding depression. If outbreeding depression is expected in the F_2 generation due in part to the disruption of favorable epistatic interactions, then tests for epistasis in traits possibly associated with fitness may provide insight into how powerful the effect of the disruption of favorable gene complexes in the F_2 generation can be. Although we did observe some strong indications of the influence of epistasis on length in these populations, we did not observe losses of fitness due to their hybridization in the F_2 generation (Chapter 1). This contrast may reflect the lack of an adult freshwater component in our tests of fitness, especially if selection for length and other salmonid fitness traits is stronger in the freshwater spawning environment than the marine growth environment. However, it is also likely that our failure to detect OBD is a consequence of low statistical power (Chapter 1).

The comparison of Q_{ST} and F_{ST} for length indicated divergent selection. Although we were unable to test rigorously for different Q_{ST} and F_{ST} values, our qualitative

assessment strongly supports a divergent selection model for length. However, because our bootstrap analysis of the F_{ST} distribution may have been foreshortened by the few loci (i.e., four, although we resampled alleles at those loci independently) and only three populations were included in our study, our comparison with Q_{ST} estimates should be interpreted cautiously (Whitlock 2008). Nonetheless, the large differences between the Q_{ST} estimate for length and the upper bounds of both F_{ST} distributions and the large body of evidence supporting local adaptation for salmonid length support our conclusion of divergent selection for length among these three coho salmon populations. The differences in length among these three coho salmon populations were described in previous variance partitioning models, which also supported the possibility of local adaptive differences (Chapter 1).

In contrast, the combined Q_{ST} analysis for meristic counts failed to show divergence among the populations. Removal of one outlier trait resulted in a composite estimate of Q_{ST} less than the median estimate of F_{ST} and the lower bound of the bootstrap confidence interval but not the Lewontin-Krakauer lower bound. The small value of Q_{ST} for meristic counts further suggests that these are highly conserved traits, but does not distinguish between physiological or genetic causes, that is, strong homeostatic canalization or either convergent selection or low variation within and among populations.

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Tables

Table 2.1.—BY97 experimental design. Parental origins abbreviated Gastineau (G), Hidden Falls (H), and Neets Bay (N). Three parental lines in boldface and six F_1 hybrid lines in italics (female parent listed first) were produced from BY94 parents, released, and recovered in 2000.

		Sire		
		Gastineau	Hidden Falls	Neets Bay
Dam	Gastineau	**GG**	*GH*	*GN*
	Hidden Falls	*HG*	**HH**	*HN*
	Neets Bay	*NG*	*NH*	**NN**

Table 2.2.—BY00 experimental design. Parental origins abbreviated Gastineau (G), Hidden Falls (H), and Neets Bay (N). Three parental control lines in boldface, six replicate F_1 hybrid lines in italics, and six F_2 hybrid lines (female parent listed first). These crosses were made from returns of the BY97 crosses (Table 1), released, and recovered in 2003.

	Sire								
Dam	GG	GH	GN	HG	HH	HN	NG	NH	NN
GG	**GGGG**				*GGHH*				*GGNN*
GH				GHHG					
GN							GNNG		
HG		HGGH							
HH	*HHGG*				**HHHH**				*HHNN*
HN								HNNH	
NG			NGGN						
NH						NHHN			
NN	*NNGG*				*NNHH*				**NNNN**

Table 2.3.—Number of BY00 fish measured for length and meristic counts in three line cross analyses.

Gastineau-Hidden Falls		Gastineau-Neets Bay		Hidden Falls-Neets Bay	
Length	Meristics	Length	Meristics	Length	Meristics
209	210	300	267	316	304

Table 2.4.—Model that best explains character means and variances in three line cross analyses based on the joint scaling test: Gastineau-Hidden Falls (G-H), Gastineau-Neets Bay (G-N) and Hidden Falls-Neets Bay (H-N). Models are abbreviated A (Additive), A-D (Additive-Dominance), A-E (Additive suggesting epistasis), A-D-E (Additive-Dominance suggesting epistasis), I (Inconclusive), and NV (No variation in a line cross). MEF is mid-eye to fork of tail; L and R are counts on the left and right sides, respectively. In the case of bilateral meristic characters, the support for any particular genetic model of divergence can also be interpreted simply as evidence low divergence among populations.

Character	G-H	G-N	H-N	Trend
Length (MEF)	A-E	A	A-D-E	A & E?
Pelvic rays (L)	A	NV	NV	A
Pelvic rays (R)	A	A	A	A
Pectoral rays (L)	A	A	A	A
Pectoral rays (R)	A	I	I	I
Branchiostegals (L)	A	A	A	A
Branchiostegals (R)	A	A	A	A
Gill Rakers				
1st arch, upper (L)	I	A	A	A
1st arch, lower (L)	A	I	A	A
1st arch, upper (R)	I	A	A	A
1st arch, lower (R)	A-D	A	A	A
2nd arch, upper (L)	A	I	A	A
2nd arch, lower (L)	A	A	A	A
2nd arch, upper (R)	A	A	A	A
2nd arch, lower (R)	I	A	A	A

Table 2.5.—Number of BY97 and BY00 offspring from multiple sibling families measured for length (L) and meristic (M) data and the sires that created them and used in the heritability analysis.

	Gastineau			Hidden Falls			Neets Bay		
	Offspring			Offspring			Offspring		
	L	M	Sires	L	M	Sires	L	M	Sires
BY97	10	10	5	7	7	3	12	12	5
BY00	27	25	6	40	38	6	60	54	3

Table 2.6.—Significance of REML (or GLM) tests of population, sire and dam effects on characters in three control populations in two generations (BY97 and BY00). Parentheses enclose GLM analysis results when REML analysis failed to converge on parameter estimates; -- indicates no trait variation. MEF is mid-eye to fork of tail, L and R are counts on the left and right sides, respectively. Tests do not include corrections for multiple tests.

	BY97		BY00		
Character	Dam	Population	Dam	Sire	Population
Length (MEF)		*			*
Pelvic Rays (L)					
Pelvic Rays (R)	--	--			
Pectoral Rays (L)					***
Pectoral Rays (R)					***
Branchiostegals (L)		**			
Branchiostegals (R)					
Gill Rakers					
1st arch, upper (L)			(**)	( )	( )
1st arch, lower (L)			(*)	( )	( )
1st arch, upper (R)	*	*			
1st arch, lower (R)			(*)	( )	( )
2nd arch, upper (L)			(*)	( )	( )
2nd arch, lower (L)					
2nd arch, upper (R)		*			
2nd arch, lower (R)			(*)	( )	( )

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table 2.7.—Significance of REML (or GLM) tests of dam and sire effects on characters in three control populations in two generations (BY97 and BY00). Parentheses enclose GLM analysis results when REML analysis failed to converge on parameter estimates; -- indicates no trait variation. MEF is mid-eye to fork of tail; L and R are counts on the left and right sides, respectively. Tests do not include corrections for multiple tests.

	Gastineau			Hidden Falls			Neets Bay		
	BY97	BY00		BY97	BY00		BY97	BY00	
Character	Dam	Dam	Sire	Dam	Dam	Sire	Dam	Dam	Sire
Length (MEF)									
VL							--	( )	( )
VR	--			--			--		
PL		( )	( )						
PR		( )	( )	(***)					
BL		( )	( )				( )		
BR		(*)	( )	( )					
UL1		(*)	( )	( )				(**)	( )
LL1		( )	( )					(*)	( )
UR1				( )					
LR1		(*)	( )	( )				( )	( )
UL2		(*)	( )		( )	(**)		(*)	( )
LL2		( )	( )					( )	( )
UR2							( )	(***)	( )
LR2		( )	( )					(*)	( )

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table 2.8.—Estimates of population divergence as measured by quantitative traits (Q_{ST}) and four microsatellite loci (F_{ST}). The second composite estimate of Q_{ST} for meristics does not include LR1. Confidence intervals for the F_{ST} estimate are from the bootstrap and the Lewontin-Krakauer (L-K) methods described in text.

		F_{ST}				
			Bootstrap 95% CI		Lewontin-Krakauer 95% CI	
Character	Q_{ST} Estimate	Estimate	Lower	Upper	Lower	Upper
Length	0.31					
All meristics	0.04					
Meristics w/o LR1	0.02					
Microsatellite loci		0.03	0.02	0.04	-0.03	0.08

Figures

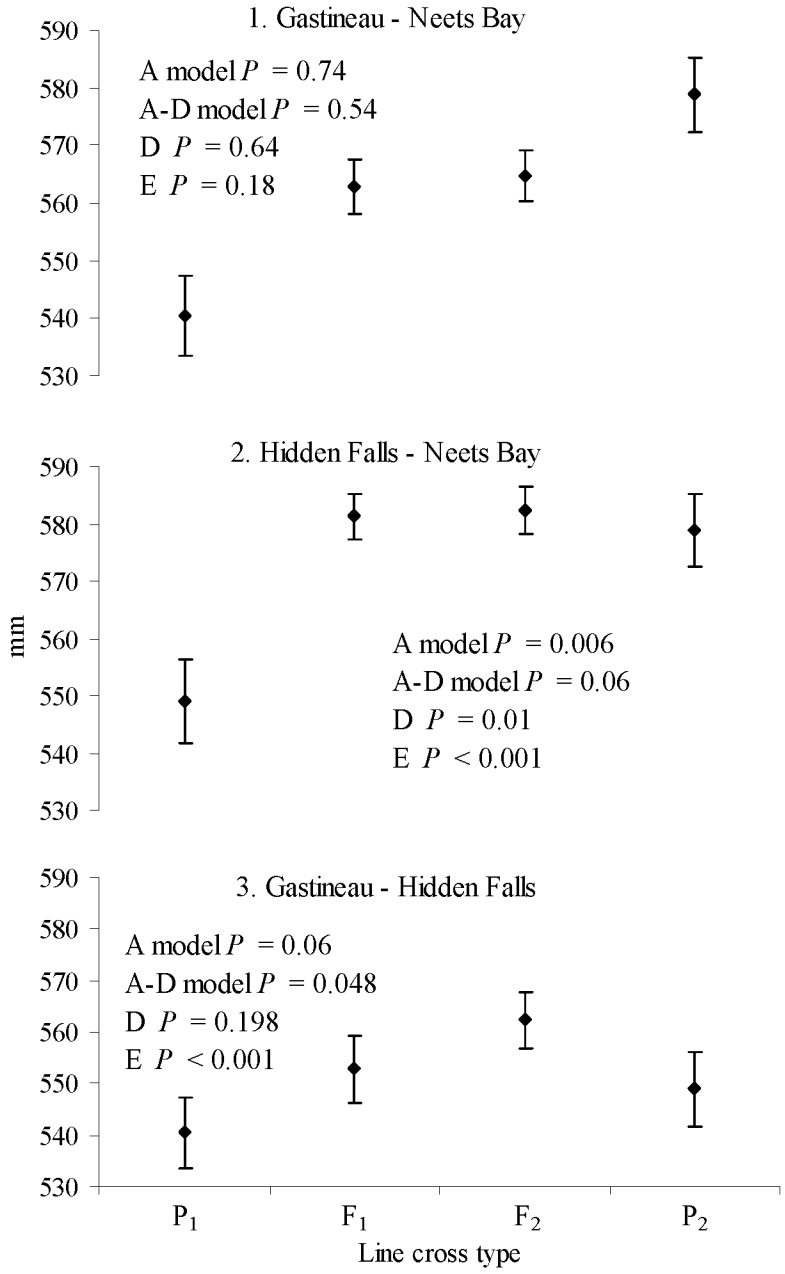

Figure 2.1.—Length means and standard errors, and associated test statistics, in three line cross analyses that represent fitted models: (A) additive, (A-D) additive-dominance suggesting epistasis, (D) tests significance of adding the dominance term, and (E) additive suggesting epistasis. Test statistics abbreviated as in text.

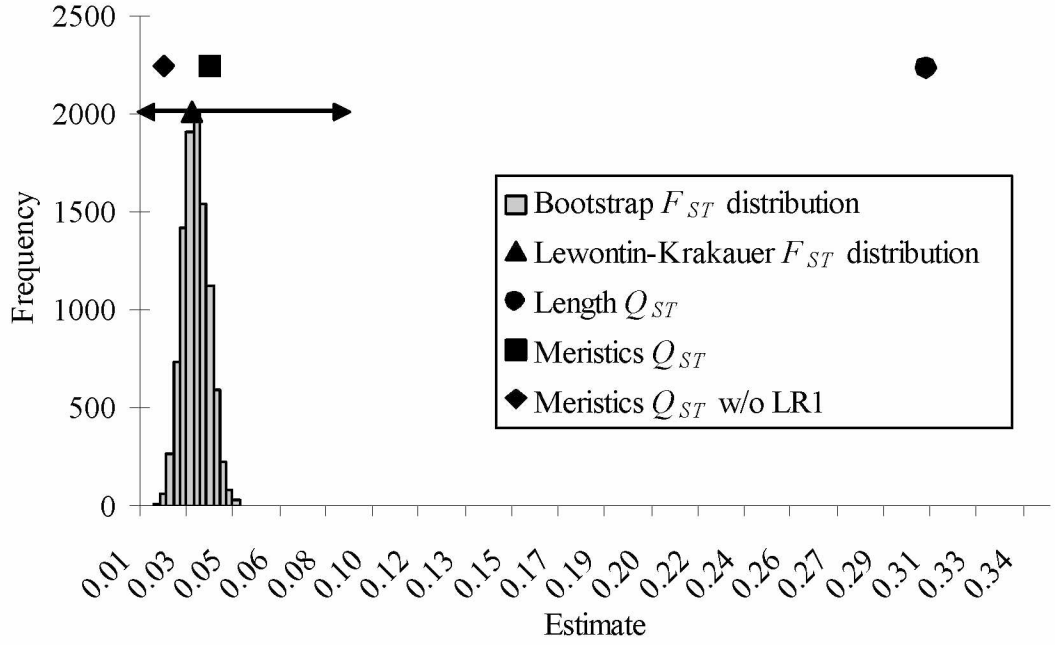

Figure 2.2.—Estimates of population divergence as measured by quantitative traits (Q_{ST}) and neutral loci (F_{ST}). The second composite estimate of Q_{ST} for meristics does not include gill raker counts of the lower right arch (LR1). Confidence intervals for the F_{ST} estimate are from the bootstrap and the Lewontin-Krakauer (L-K) methods described in text.

General Conclusions

We observed no losses in fitness resulting from the hybridization of the coho salmon populations included in this study, but the power of the tests was low. It may be that these populations are not sufficiently divergent for losses in fitness to result from hybridization. It may also be that the power of our tests was too low to detect real biological differences in fitness that resulted from outbreeding, or that other measures of fitness may have better detected fitness changes. Regardless, the environmental conditions and evolutionary history experienced by the coho populations in this study are unique to this study and caution should be exercised in extending these results to other situations.

We observed little genetic variance for the quantitative traits measured in this study, but again the power of these tests was low because relatively few sires were included in our heritability analysis. The nature of the variation for length differed from that of the bilateral meristics that we measured because it exhibited substantial variation both within and among populations, showed indications of epistatic gene action, and appeared to be under divergent selection among the populations we measured. In contrast, the meristic traits that were measured appeared to be strongly canalized: they exhibited very little variability, and in the line cross examples, they were best explained by additive gene action. However, the process of fitting the additive model resulted from failure to reject the null hypothesis. Consequently, the traits may also be explained by low divergence among populations; and comparisons of Q_{ST} and F_{ST} values suggested that they were not influenced by selection, although they probably encountered strong stabilizing selection in their evolutionary history.

This study incorporated population and quantitative genetic analyses in an uncommon synthesis. The results of the line cross analysis suggested we might have observed significant genetic variance given adequate power and suggest the potential for fitness losses due to the disruption of coadapted gene complexes. Future studies that incorporate both approaches would benefit from increasing the number of sires used for

the crosses. It would also be useful to evaluate experimental populations in each source environment to assess genotype by environment interactions, although the logistics of such an experiment would be challenging.

Appendix

Table A1.—Number of families, dams and sires that produced the families and offspring for BY97 crosses.

	Number of:			Number of offspring:				
Cross	Families	Dams	Sires	Total	Average	Min.	Max.	Std. Dev.
GG	12	12	12	18	1.50	1	3	0.67
GH	13	13	13	20	1.54	1	3	0.66
GN	18	18	18	25	1.39	1	4	0.85
HG	18	18	18	25	1.39	1	3	0.70
HH	13	13	13	17	1.31	1	3	0.63
HN	18	18	18	26	1.44	1	3	0.70
NG	15	15	15	31	2.07	1	5	1.22
NH	19	19	19	32	1.68	1	3	0.75
NN	15	15	15	24	1.60	1	4	0.91
Mean	15.67	15.67	15.67	24.22	1.55			
SD	2.65	2.65	2.65	5.24	0.23			

Table A2.—Number of families, dams and sires that produced the families and offspring for BY00 crosses.

	Number of:			Number of offspring:				
Cross	Families	Dams	Sires	Total	Average	Min.	Max.	Std. Dev.
GGGG	12	9	7	43	3.58	0	10	3.00
GGHH	10	9	6	54	5.40	1	13	3.78
GGNN	9	9	3	75	8.33	0	16	4.82
GHHG	10	5	6	80	8.00	1	19	4.99
GNNG	12	12	11	75	6.25	0	13	3.65
HGGH	11	10	9	48	4.36	1	10	2.77
HHGG	9	7	6	29	3.22	0	10	3.19
HHHH	11	7	6	59	5.36	1	11	2.77
HHNN	7	7	3	63	9.00	4	15	3.51
HNNH	11	11	11	68	6.18	0	13	3.82
NGGN	12	11	7	79	6.58	1	10	2.94
NHHN	12	12	7	71	5.92	0	11	3.55
NNGG	10	10	7	49	4.90	3	9	2.13
NNHH	10	10	6	81	8.10	4	13	3.07
NNNN	12	11	3	69	5.75	2	13	3.77
Mean	10.53	9.33	6.53	62.87	6.06			
SD	1.46	2.06	2.47	15.48	1.72			

Table A3.—Coefficients and probabilities of length regressions on bilateral characters in both the generation-specific and stock-specific data sets. L and R are counts on the left and right sides, respectively. [a] indicates significant result after correcting for multiple tests with a sequential Dunn-Sidak correction for $k = 14$ tests (14 bilateral traits).

	Generation-specific		Stock-specific	
Character	β	P	β	P
Pelvic rays (L)	0.000	0.62	0.000	0.68
Pelvic rays (R)	0.000	0.23	0.000	0.85
Pectoral rays (L)	0.000	0.40	-0.001	0.03
Pectoral rays (R)	-0.001	0.08	-0.001	0.02
Branchiostegals (L)	0.002	0.01	0.000	0.71
Branchiostegals (R)	0.001	0.05	0.000	0.64
Gill rakers				
1st arch, upper (L)	0.001	0.01	0.000	0.38
1st arch, lower (L)	0.000	0.56	0.001	0.28
1st arch, upper (R)	0.001	0.04	0.000	0.30
1st arch, lower (R)	-0.001	0.37	0.002	0.01
2nd arch, upper (L)	0.000	0.29	-0.001	0.10
2nd arch, lower (L)	0.000	0.81	0.001	0.03
2nd arch, upper (R)	0.001	0.05	-0.002	0.00[a]
2nd arch, lower (R)	0.000	0.94	0.001	0.22

Table A4.—Pearson correlation coefficients among BY00 character data. Characters abbreviated as in text. Tests were corrected for multiple tests with a Dunn-Sidak correction.

	MEFL	VL	VR	PL	PR	BL	BR	UL1	LL1	UR1	LR1	UL2	LL2	UR2
VL	-0.02													
VR	0.05	0.43[c]												
PL	-0.04	0.20[c]	0.20[c]											
PR	-0.08	0.19[c]	0.21[c]	0.67[c]										
BL	0.11	0.03	0.03	0.06	0.05									
BR	0.09	0.00	0.09	0.08	0.04	0.48[c]								
UL1	0.11	-0.01	0.02	0.02	0.00	0.13	0.11							
LL1	0.03	-0.05	-0.05	0.02	0.03	0.06	0.06	0.22[c]						
UR1	0.09	0.01	0.01	-0.04	-0.01	0.15[a]	0.15[a]	0.58[c]	0.20[c]					
LR1	-0.04	-0.11	-0.07	-0.01	0.02	0.09	0.09	0.24[c]	0.40[c]	0.27[c]				
UL2	0.05	0.10	0.05	0.09	0.08	0.15[a]	0.14[a]	0.30[c]	0.21[c]	0.29[c]	0.25[c]			
LL2	0.01	-0.05	-0.05	0.00	-0.01	0.16[b]	0.09	0.20[c]	0.25[c]	0.18[c]	0.25[c]	0.23[c]		
UR2	0.09	0.09	0.00	0.07	0.06	0.19[c]	0.15[b]	0.32[c]	0.16[b]	0.31[c]	0.23[c]	0.61[c]	0.28[c]	
LR2	0.00	0.00	0.05	0.01	-0.01	0.08	0.04	0.20[c]	0.32[c]	0.19[c]	0.29[c]	0.18[c]	0.35[c]	0.19[c]

[a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$.

Table A5.—Pearson correlation coefficients among character data from parental controls from all broodyears. Characters abbreviated as in text. Tests were corrected for multiple tests with a Dunn-Sidak correction.

	MEFL	VL	VR	PL	PR	BL	BR	UL1	LL1	UR1	LR1	UL2	LL2	UR2
VL	-0.01													
VR	0.01	0.43[c]												
PL	-0.10	0.17	0.12											
PR	-0.09	0.13	0.12	0.51[c]										
BL	0.03	0.05	0.11	0.14	0.16									
BR	0.00	0.14	0.16	0.13	0.10	0.53[c]								
UL1	-0.03	0.08	0.11	-0.09	-0.02	-0.01	0.05							
LL1	0.06	0.02	0.02	-0.02	0.07	0.08	0.03	0.01						
UR1	-0.04	0.10	0.06	-0.05	-0.02	-0.03	0.09	0.63[c]	-0.03					
LR1	0.14	-0.01	0.01	-0.01	0.01	0.09	0.04	-0.10	0.54[c]	-0.1				
UL2	-0.07	0.02	0.06	0.01	-0.02	0.11	0.14	0.52[c]	0.02	0.43[c]	-0.11			
LL2	0.10	-0.01	-0.02	0.00	0.03	0.13	0.04	-0.03	0.42[c]	-0.11	0.43[c]	-0.04		
UR2	-0.17	0.00	0.01	0.03	0.00	0.08	0.08	0.45[c]	0.00	0.45[c]	-0.08	0.63[c]	-0.05	
LR2	0.06	0.02	-0.01	0.05	0.04	0.13	-0.02	-0.01	0.42[c]	-0.06	0.42[c]	-0.06	0.45[c]	-0.11

[a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$.

Table A6.—Significance of GLM tests of stock-specific model effects in control populations of BY94, BY97 and BY00. Effects are abbreviated Year (Y), Population (P) and Sex (S). MEF is mid-eye to fork of tail; L and R are counts on the left and right sides, respectively. Tests do not include corrections for multiple tests.

Character	Y	P	S	Y*P	Y*S	P*S	Y*P*S
Length (MEF)	***	***	***	***	**	**	
Pelvic rays (L)				***		**	*
Pelvic rays (R)							
Pectoral rays (L)	*	**		***			**
Pectoral rays (R)	**	*		***			
Branchiostegals (L)	***						
Branchiostegals (R)	***						
Gill rakers							
1st arch, upper (L)	***						
1st arch, lower (L)	***	*					
1st arch, upper (R)	***						
1st arch, lower (R)	***	***	*				
2nd arch, upper (L)	***					*	
2nd arch, lower (L)	***	*					
2nd arch, upper (R)	***						
2nd arch, lower (R)	***			*			*
CFA1	***						
CFA2	**						
CFA3	**						
CFA5	**						

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table A7.—Significance of GLM tests of hybrid-cross model effects in comparisons of two control populations and their pooled hybrids in BY00. Results of tests of controls and F_1 hybrids precede slash (i.e. */), results of tests of controls and F_2 hybrids follow slash (i.e. /*). MEF is mid-eye to fork of tail; L and R are counts on the left and right sides, respectively. Tests do not include corrections for multiple tests.

	GG-HH			GG-NN			HH-NN		
Character	Cross	Sex	C*S	Cross	Sex	C*S	Cross	Sex	C*S
Length (MEFL)	/*	/***	***/	*/**	***/***		**/**	***/***	
Pelvic rays (L)									
Pelvic rays (R)				*/			/*		
Pectoral rays (L)		*/		**/*			***/***		
Pectoral rays (R)	/*			**/**			***/***		
Branchiostegals (L)									
Branchiostegals (R)									
Gill rakers									
1st arch, upper (L)		*/					*/		
1st arch, lower (L)	/*	*/			/*				
1st arch, upper (R)								/*	
1st arch, lower (R)	/*								
2nd arch, upper (L)									
2nd arch, lower (L)									
2nd arch, upper (R)					*/				
2nd arch, lower (R)									
CFA1						**/			*/
CFA2	*/					**/			*/
CFA3						**/			*/
CFA5					/*	*/			

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table A8.—Significance of chi-square tests for model fit of models including additive (A) and additive and dominance (A+D) effect parameters, of dominance variation (DV) in improving model fit, and of epistasis (E; delta ratio method) in characters of three line cross analyses. Significant results indicate poor model fit for A and A+D, significant model improvement by DV inclusion, and the presence of epistasis, respectively. Populations abbreviated as in Table 1. MEF is mid-eye to fork of tail; L and R are counts on the left and right sides, respectively. -- indicates no trait variation in a line's character. Tests do not include corrections for multiple tests.

	GG-HH				GG-NN				HH-NN			
Character	A	A+D	D	E	A	A+D	D	E	A	A+D	D	E
Length (MEF)		*		***					**		*	***
Pelvic Rays (L)					--	--	--		--	--	--	
Pelvic Rays (R)							*					
Pectoral Rays (L)										*		
Pectoral Rays (R)			*		*	*			*	**		
Branchiostegals (L)												
Branchiostegals (R)												
Gill Rakers												
1st arch, upper (L)	**	*	**									
1st arch, lower (L)					**	**						
1st arch, upper (R)	**	*										
1st arch, lower (R)	*											
2nd arch, upper (L)					*	*						
2nd arch, lower (L)												
2nd arch, upper (R)												
2nd arch, lower (R)	*	**										

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

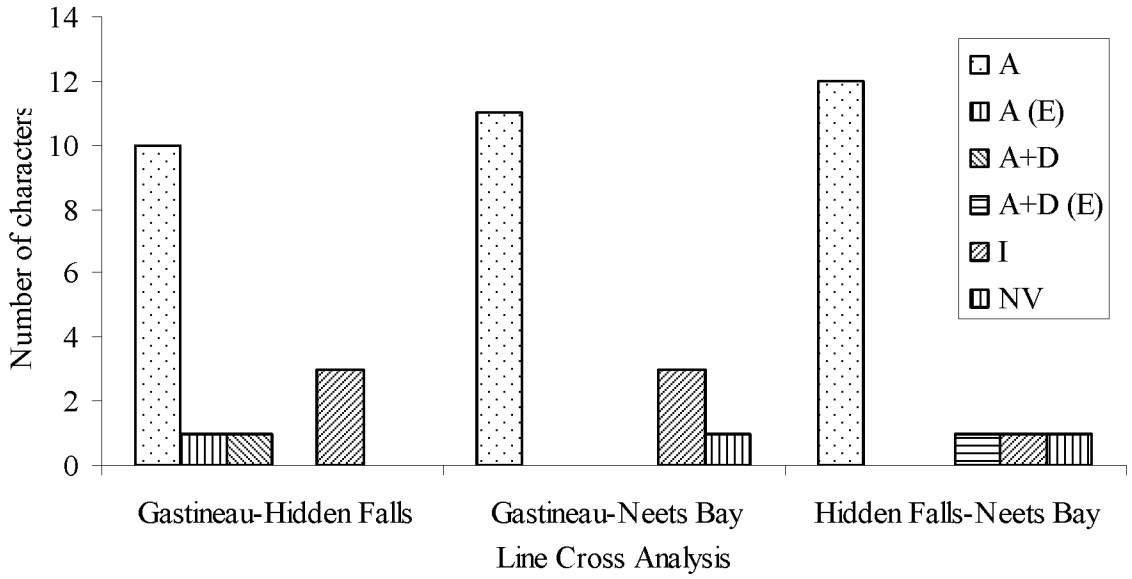

Figure A1.—Number of characters best explained by an additive (A), additive suggesting epistasis (A (E)), additive and dominance (A+D), or additive and dominance suggesting epistasis (A+D (E)) model in three line cross analyses. I indicates an inconclusive result; NV indicates analysis failure due to zero variation in one line of analysis.